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(54) Title: POLYPEPTIDES CAPABLE OF FORMING ANTIGEN BINDING STRUCTURES WITH SPECIFICITY FOR THE RHESUS D ANTIGENS, THE DNA ENCODING THEM AND THE PROCESS FOR THEIR PREPARATION AND USE

#### (57) Abstract

Polypeptides capable of forming antigen binding structures specific for Rhesus D antigens include the sequences indicated in the figures 1a to 16b. The obtained polypeptides, being Fab fragments, may be used directly as an active ingredient in pharmaceutical and diagnostic compositions. The Fab and their DNA sequences can also be used for the preparation of complete recombinant Anti-Rhesus D antibodies. Useful in pharmaceutical and diagnostic compositions.

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# Polypeptides capabl of forming antigen binding structures with specificity for the Rhesus D antigens, th DNA ncoding th m and the process for their preparation and use

This invention relates to polypeptides forming antigen binding
structures with specificity for Rhesus D antigens and especially to Fab
molecules with specificity for the Rhesus D antigen. The invention also
relates to their application to provide pharmacological and diagnostic
compositions. The above Fab fragments when genetically engineered to be
part of complete antibodies are useful for the prophylaxis of hemolytic
disease of the newborn (HDN). This invention provides the novel DNA and
amino acid sequences of the above polypeptides.

Thus, the antibodies can be used for the protection of Rhesus negative women before or immediately after the birth of a Rhesus positive child to prevent HDN in subsequent pregnancies.

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The invention also includes the application of the said Fab molecules either alone or in combination with Fc constant regions as complete antibodies for the purposes of treating other illnesses which might benefit from anti-Rhesus D immunoglobulin e.g. treatment of idiopathic thrombocytopenic purpura (ITP).

In addition anti-Rhesus D immunoglobulin can be used after mistransfusions of Rhesus positive blood to Rhesus negative recipients in order to prevent sensitization to the Rhesus D antigen. Further the invention relates to the application of these Fab fragments and antibodies as diagnostic reagents.

HDN is the general designation for hemolytic anemia of fetuses and newborn babies caused by antibodies of the mother. These antibodies are directed against antigens on the surface of the fetal erythrocytes. These antigens can belong to the Rhesus, ABO or other blood group systems.

The Rhesus blood group system includes 5 major antigens: D, C, c, E and e (Issitt, P.D., Med. Lab. Sci. 45:395, 1988). The D antigen is the most important of these antigens as it is highly immunogenic eliciting anti-Rhesus D antibodies during Rhesus incompatible pregnancies and following transfusion of Rhesus incompatible blood. The D antigen is found in approximately 85% of Caucasians in Europe and those individuals are said to

be Rhesus positive. Individuals lacking the D antigen are called Rhesus negative. The expression of the D antigen can vary due to either low antigen density, hereafter known as weak D or D<sup>u</sup>, or due to partial antigenicity, hereafter known as partial D antigens.

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The Rhesus D antigen, a membrane protein of the erythrocyte, has recently been cloned and its primary structure described (Le Van Kim, C., et al., PNAS 89:10925, 1992). Modeling studies suggest that the Rhesus D antigen has 12 transmembrane domains with only very short connecting regions extending outside the cell membrane or protruding into the cytoplasm.

The partial D phenotypes were first identified in people who carried D antigen on their red cells but who had an alloanti-D in their sera (Rose, R. R. and Sanger, R., Blood groups in man, Blackwell Scientific, Oxford, U.K. 1975; Tippett, P. et al., Vox Sanguinis. 70:123, 1996). This can be explained by regarding the D antigen as a mosaic structure with at least 9 different epitopes (epD1 to epD9). Thus in some D variant people the red cells lack part of this mosaic and antibodies are made to the missing D epitopes. Rhesus positive individuals that make antibodies against partial D antigens have been classified into six main different categories (DII to DVII) each having a different abnormality in the D antigen. More recently it has been shown that these D categories gave different patterns of reaction when tested against panels of human monoclonal anti-D antibodies (Tippett, P., et al., Vox Sanguinis. 70:123, 1996). The different reaction patterns identified the 9 epitopes and so define the different partial D categories. The number of epitopes present on the D antigen varies from one partial D category to another with the  $D^{VI}$  category expressing the least, epD3, 4 and 9. The  $D^{VI}$ category is clinically important as a DVI woman can be immunized strongly enough to cause hemolytic disease of the newborn.

The prophylactic efficacy of anti-RhD IgG for prevention of hemolytic disease of the newborn is well established and has been in routine use for many years. As a result this severe disease has become a rarity. Nevertheless the underlying cause of the disease, i.e. RhD incompatibility between a RhD negative mother carrying a RhD positive child still remains and thus requires a continual supply of therapeutic anti-RhD IgG.

In recent years the assurance of a continual supply of anti-RhD lgG has become an increasing problem. The pool of available hyperimmune

serum from alloimmunized multiparous Rhesus negative women has drastically decreased due to the success of prophylactic anti-RhD. Thus the current methods of production require repeated immunization of an increasingly reluctant pool of donors for the production of high titer antiserum (Selinger, M., Br. J. Obstet. Gynaecol. 98:509, 1991). There are also associated risk factors and technical problems such as the use of Rhesus positive red blood cells for repeated immunization carrying the risk of transmission of viral diseases like hepatitis B, AIDS and other as yet unknown viruses (Hughes-Jones, N.C., Br. J. Haematol. 70:263, 1988). Therefore an alternative method for production of anti-RhD antibodies is required.

In the past few years various alternative sources of hyperimmune serum have been tried but all are associated with disadvantages. Epstein Barr Virus (EBV) transformation of lymphocytes creating B lymphoblastoid cell lines that secrete specific antibody including against the Rhesus D antigen (Crawford et al., Lancet. 386:Feb.19th, 1983) are unstable and require extensive cloning. Also due to the low transformation efficiencies (1-3% of B cells) only a restricted range of antibody specificities can be obtained from the potential repertoire. Additionally it seems that mice do not respond to the Rhesus D antigen and thus no murine monoclonal antibodies are available which could be used for producing chimaeric or humanised antibodies. Until recently the only other alternative was production of human antibodies by the hybridoma technique which was also restricted by the lack of a suitable human myeloma cell fusion partner (Kozbor, D. and Roder, J.C., Immunol. Today, 4:72, 1983).

It is thus the object of the present invention to provide Fab fragments having a reactivity against the Rhesus D antigen as well as complete antibodies comprising the Fab fragments which are free from the above mentioned drawbacks.

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In the last few years the technique of repertoire cloning and the construction of phage display libraries has opened up new possibilities to produce human antibodies of defined specificity (Williamson, R.A. et al., PNAS 90:4141, 1993). These methods were thus applied to the preparation of polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens, especially of Fab fragments having an activity against Rhesus D and partial D antigens.

The generation of human antibodies by repertoire cloning as described in recent years (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991) is based on isolating mRNA from peripheral B cells. This method offers the tools to isolate natural antibodies. 5 autoantibodies or antibodies generated during the course of an immune response (Zebedee, S.L., et al., PNAS 89:3175, 1992; Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). This method relies on constructing a recombinant antibody library from a particular donor starting from the mRNA coding for immunoglobulin (Ig) molecules. As only the peripheral blood lymphocytes (PBL) can be isolated from a donor the chances of finding specific antibody producing B cells in the periphery are increased if an individual is boosted with the desired antigen shortly before harvesting the PBL (Persson, M.A.A., et al., PNAS 88:2432, 1991). The total RNA is then isolated and the mRNA of the Ig repertoire can be cloned using Ig specific primers in the polymerase chain reaction (PCR) followed by the co-expression of heavy and light chains of the Ig molecule on the surface of a filamentous phage particle thereby forming an "organism" that in analogy to a B cell can bind to an antigen. In the literature this method is also known as the combinatorial approach as it allows the independent combining of heavy and light chains to form a functional Fab antibody fragment attached to one of the tail proteins, called plll, of a filamentous phage. Phages carrying the Fab molecules (hereafter known as Phab particles) are selected for the desired antigen specificity, by a process known as bio-panning. The antigen can be applied to a solid support, specific Phab bind to the antigen whilst non specific Phab are washed away and finally the specific Phab are eluted from the solid support. The specific Phab are then amplified in bacteria, allowed to re-bind to the antigen on the solid support and the whole process of bio-panning is repeated.

The successive rounds of panning and amplification of selected Phab in bacteria result in an enrichment of specific Phab that can be seen from a rise in titer of colony forming units (cfu) plated out after each round of panning. Our previous experience and published data indicate that specific phage can usually be detected after 4 to 6 panning rounds (Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). In the above cited related art there is, however, no hint that the indicated steps can be used for a successful preparation of Fab fragments of anti-Rh D antibodies.

In the appended figures 1a to 16b; DNA sequences coding for variable regions (V regions) of anti Rh D Fab fragments and the corresponding polypeptide sequences are disclosed.

Fig. 17 shows the pComb3 expression system used according to the present invention.

Figs. 18 and 19 show the separate preparation of genes of the heavy and light chains of the complete antibody according to the description in example 6.

Subjects of the present invention are polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens according to the definition of claim 1. The table in claim 1 refers to the appended figures. The identification number for each sequence is given. The locations of the Rhesus D specific CDR1 (complementarity determining region 1), CDR2 and CDR3 regions are indicated in the figures and according to base pair number in the table of claim 1. Preferred polypeptides according to the invention are anti-Rhesus D antibodies which include the variable regions of the heavy and light chains according to the sequences given in Figs. 1a -16b. The Figs. 1a, 2a, ... 16a are related to the variable regions of the heavy chain and the Figs. 1b, 2b, ... 16b are related to the variable regions of the light chain.

Further subjects of the present invention are the DNA sequences coding for antigen binding polypeptides according to the definition of claim 6. Prefered DNA sequences are those coding for variable regions of Fab fragments of anti-Rh D antibodies according to the Figs. 1a -16b. The Figs. 1a, 2a, ... 16a are related to the heavy chain and the Figs. 1b, 2b, ... 16b are related to the light chain.

A further subject of the present invention is a process for preparing recombinant Fab polypeptides according to the definition in claim 11.

A further subject of the present invention is a process for the selection of recombinant polypeptides according to claim 12.

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Further subjects of the present invention are anti-Rh D antibodies according to the definition of claim 14, preferably anti-Rh D immunoglobulin molecules comprising the heavy and light chain variable regions according to

the Figs. 1a to 16b combined with known heavy and light chain constant regions.

Further subjects of the present invention are pharmaceutical and diagnostic compositions comprising polypeptides, anti-Rh D antibodies or Fab fragments according to the invention.

The total re-amplified Phab population obtained after each panning can be tested for specificity using various methods such as ELISA and immunodot assays. It is also defined by the nature of the antigen e.g. anti-Rhesus D Phabs are detected by indirect haemagglutination using a rabbit anti-phage antibody or equivalent Coombs reagent as the cross linking antibody. Once a total Phab population has been identified as positive for the desired antigen, individual Phab clones are isolated and the DNA coding for the desired Fab molecules is sequenced. Individual Fab can then be produced by use of the pComb3 expression system which is illustrated in Fig. 16. In this system the gIII gene, coding for the tail protein pIII, is cut out from the phagemid vector pComb3. This allows production of soluble Fab in the bacterial periplasm. Such individual Fab fragments can then be tested for antigen specificity.

The phage display approach has also been used as a means of rescuing monoclonal antibodies from unstable hybridoma cell lines. This has been reported for anti-Rhesus D antibodies (Siegel, D.L. and Silberstein, L.E., Blood. 83:2334, 1994; Dziegiel, M. et al., J. Immunol. Methods. 182:7, 1995). A phage display library constructed from non-immunized donors has also been used to select Fv fragments (i.e. variable regions of heavy and light chains, V<sub>H</sub> and V<sub>L</sub>) specific for human blood group antigens which included one Fv fragment reacting against the Rhesus D antigen (Marks, J.D. et al., Biotechnology. 11:1145, 1993).

Important considerations when constructing combinatorial libraries are the source of cells used for RNA extraction and the nature of the antigen used for panning. Therefore, this invention uses a hyperimmune donor who was boosted i.v. with Rhesus D\* red blood cells (rbc). The PBL of the donor were harvested at +5 and +18 days after the i.v. boost and were used to construct 2 combinatorial libraries hereafter known as library D1 (LD1) and library D2 (LD2) respectively. Double immunofluorescence analysis of the harvested PBL, using the markers CD20 and CD38 for pan B cells and

lymphoblastoid cells respectively, showed a higher than normal percentage of lymphoblastoid B cells, of plasma cell morphology. The high number of plasma cells found in the peripheral blood is most unusual as normally there are less than 1% in the periphery and probably indicates that the donor had a high percentage of circulating B cells with specificity for the Rhesus D antigen.

After construction of the library, the selection of Phabs specific for the Rhesus D antigen was achieved by bio-panning on fresh whole rbc of phenotype R1R1 (CDe/CDe) i.e. the reference cells used for Rhesus D typing. This was necessary since the Rhesus D antigen, an integral membrane protein of 417 amino acids (Le Van Kim, C. et al, PNAS 89:10925, 1992), loses its immunogenicity during purification (Paradis, G. et al, J. Immunol. 137:240, 1986) and therefore a chemically purified D antigen cannot be bound to a solid phase for selection of immunoreactive Phabs as for other antigen specificities previously selected in this system (Vogel, M. et al., Eur.J. Immunol. 24:1200, 1994). Modelling studies have suggested that only very short connecting regions of the Rhesus D antigen extend outside the cell membrane or protrude into the cytoplasm (Chérif-Zahar, B. et al, PNAS 87:6243, 1990). Thus the parts of the RhD antigen visible to antibodies are relatively restricted and may be under conformational constraint. This aspect of the Rhesus D antigen becomes even more important when considering selection of Phabs with reactivity against the partial D phenotypes which essentially lack certain defined epitopes of the D membrane protein (Mouro, I. et al, Blood. 83:1129, 1994).

Furthermore, since whole rbc do not only express the D antigen, a series of negative absorptions had to be performed on Rhesus D negative rbc in order to absorb out those Phabs reacting with the other antigenic proteins found on the rbc.

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This panning procedure performed on Phabs coming from both LD1 and LD2 librairies resulted in the isolation of 6 different Fab producing clones from library LD1, 8 different Fab producing clones from library LD2 and 2 Fab producing clones from the pooled libraries LD1 and LD2.

The nomenclature and the figures where the sequences are listed are given in table 1.

Table 1

LIBRARY LD1 Clone No.	V <sub>H</sub> - Sequence Figure	V <sub>L</sub> - Sequence Figure	LIBRARY LD2 Clone No.	V <sub>H</sub> - Sequence Figure	V <sub>L</sub> - Sequence Figure
LD1-40	1a	1b	LD2-1	6a	6b
LD1-52	2a	2b	LD2-4	7a	7b
LD1-84	3a	3b	LD2-5	8a	8b
LD1-110	4a	4b	LD2-10	9a	9b
LD1-117	5a	5b	LD2-11	10a	10b
			LD2-14	11a	11b
			LD2-17	12a	12b
			LD2-20	13a	13b

The above Fab clones show exclusive reactivity against the Rhesus D antigen, 3 of 5 D<sup>u</sup> rbc tested and agglutinating reactivity against the Partial D phenotypes as follows: Rh33, DIII, DIVa, DIVb, DVa, DVII,.

However, using the above mentioned R1R1 rbc for panning of the Phabs, no clones were isolated which reacted against the Partial DVI phenotype. As the serum of the original hyperimmune donor tested at the time of construction of the recombinant library, was known to react against the DVI phenotype the recombinant library should also contain the anti-DVI specificity.

In order to select for the DVI reactivity the panning conditions were changed in that different cells were used. A special donor whose rbc had been typed and were known to express the Partial DVI phenotype was used as the source of cells for re-panning the LD1 and LD2 libraries. This second series of pannings was essentially performed in the same way as the first series except for the substitution of DVI rbc for R1R1 rbc and the addition of bromelase treatment to the DVI rbc. The DVI phenotype expresses the least number of Rhesus D epitopes and it is therefore difficult to make antibodies against it. It has been reported that only 15% of unselected polyclonal anti-D and 35% of selected anti-D made by Rhesus D negative subjects reacted with DVI+ cells (Mouro, I. et al, Blood. 83:1129, 1994). Bromelase treatment which removes N- acetylneuraminic acid (sialic acid) from the rbc membrane, was performed in order to render the Rhesus DVI epitopes more accessible during the panning with the pre-absorbed Phabs.

This second series of pannings on the LD1 library resulted in 1 Fab producing clone LD1-6-17. The nomenclature is given in table 2.

Table 2

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LIBRARY LD1	V <sub>H</sub> -Sequence figure	V <sub>L</sub> -Sequence figure	
Clone No: LD1-6-17	14a	14b	

However this clone was reacting with Rhesus alleles C and E and showing a false positive reaction with DVI positive rbc. This was also due to the phenotype of the DVI donor (Cc DVI ee) who expressed the C allele which was not absorbed out by the Rhesus negative rbc (ccddee).

Thus a third series of pannings on a pool of the LD1 and LD2 libraries was performed using different rbc for the absorption phase. After 6 rounds of panning using both bromelase treated and non treated rbc for both the absorption steps and the elution from DVI positive rbc a total population of Phabs was obtained which reacted exclusively with rbc of phenotype R1R1 (CCDDee) and 2 different donors expressing the DVI variant.

This third series of pannings on the LD1 and LD2 librairies resulted in 2 Fab producing clones reacting with DVI+ rbc. The nomenclature is given in table 3.

Table 3

LIBRARY LD1/LD2	V <sub>H</sub> -Sequence figure	V <sub>L</sub> -Sequence figure
Clone No: LD1/2-6-3	15a	15b
Clone No: LD1/2-6-33	. 16a	16b

Thus a total of 16 different anti-Rhesus D Fab clones have been isolated. The DNA from these clones has been isolated and sequenced using Fluorescent Cycle Sequencing on an ABI 373A Sequencing System. The nucleotide and corresponding amino acid sequences of the said Fab clones form the basis of this invention.

Sequence analysis has revealed that several clones were isolated bearing the same  $V_H$  gene segment but different  $V_L$  gene segments. This is

the case for the two clones LD2-1 and LD2-10, for the two clones LD2-4 and LD2-11, and for the three clones LD2-14, LD1/2-6-3 and LD1/2-6-33, respectively.

The DNA sequences obtained and Fab fragments are useful for the preparation of complete antibodies having an activity against the Rhesus D antigen. Suitable expression systems for such antibodies are mouse myeloma cells or chinese hamster ovary cells.

The examples which follow explain the invention in detail, without any restriction of the scope of the invention.

Example 1 describes the construction of 2 combinatorial librairies; especially the aforementioned LD1 and LD2 libraries.

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Example 2 describes a series of pannings using R1R1 rbc on the said LD1 and LD2 libraries in detail.

Example 3 describes a series of pannings using both bromelase and non bromelase treated rbc for absorption and bromelase treated DVI positive rbc using a pool of the said LD1 and LD2 librairies.

Example 4 describes an indirect haemagglutination assay using a rabbit anti-phage antibody, as an equivalent Coombs reagent, to monitor the enrichment and specificity of Rhesus D specific Phabs after panning.

Example 5 describes the preparation and purification of Fab antibody fragments for application as diagnostic reagents.

Example 6 describes the preparation of complete anti-Rhesus D immunoglobulins using the sequences of the present invention.

### Example 1

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## Construction of the recombinant LD1 and LD2 libraries

### a) Source of the lymphocytes

A male adult who was a member of the volunteer pool of 5 hyperimmune Rhesus D donors was given an i.v. boost of 2 ml of packed rbc from a known male donor of blood group O RhD\*. The PBL were harvested at +5 and +18 days after the boost and the mononuclear cells (MNC) isolated by Ficoll gradient centrifugation (Lymphoprep, Pharmacia, Milwaukee, WI). The results of donor lymphocyte analysis of day +5 are given in table 4. The +5 day MNC were used directly for RNA preparation using a phenol-chloroform guanidinium isothiocyanate procedure (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162:156, 1987). The +18 day MNC were first cultured for 3 days in RPMI-1640 medium (Seromed, Basel) containing 10<sup>3</sup> U/ml of IL-2 (Sandoz Research Center, Vienna, Austria) and 10 µg/ml of pokeweed mitogen (PWM; Sigma L9379, Buchs, Switzerland) before extracting RNA.

Table 4 Immunofluorescence analysis of donor lymphocytes +5 days after rbc i.v. boost

Cell surface antigen	% Positive cells	Cell surface antigen	% Positive cells	
CD20	15	CD8	12	
· CD38	20	CD25	7.6	
CD20/38	. 15	CD57	12.5	
•	47	CD14	6	
CD3	34	HLA-DR	18	
CD4	34	110(0)		

## b ) Construction of Library

Two separate libraries were constructed called LD1 and LD2 (as detailed above) corresponding to the cells harvested at +5 days and +18 days (finally +21 days including the +3 days PWM stimulation) after the i.v. boost respectively. Total RNA was then prepared from these cells using a phenolchloroform guanidinium isothiocyanate method. From this RNA, 10  $\mu g$  were

used to make cDNA using an oligo(dT) primer (400 ng) and reverse transcribed with M-MuLV reverse transcriptase according to the conditions specified by the supplier (Boehringer Mannheim Germany). PCR amplification was performed as described in Vogel, M. et al., E.J. of Immunol. 24:1200, 1994. Briefly, 100 µl PCR reaction contained Perkin-Elmer buffer with 10 mM MgCl<sub>2</sub>, 5 μl cDNA, 150 ng of each appropriate 5' and 3' primer, all four dNTP at 200  $\mu\text{M}$  each and 2 U/ml Taq Polymerase (Perkin Elmer, NJ). The PCR amplification of the heavy and light chains of the Fab molecule was performed separately with a set of primers from Stratacyte (details given below). For the 10 heavy chain six upstream primers were used that hybridize to each of the six families of the  $V_{\text{H}}$  genes whereas one kappa and one lambda chain primer were used for the light chain. The downstream primers were designed to match the hinge region of the constant domains  $\gamma 1$  and  $\gamma 3$  for the heavy chain. For the light chain the downstream primers were matched to the  $3^\prime$  end of kappa and lambda constant domains. The heavy and light chain PCR products were pooled separately, gel purified and cut with Xho1/Spe1 and Sac1/ Xba1 restriction enzymes (Boehringer Mannheim), respectively. After digestion the PCR products were extracted once with phenol: chloroform: isoamylalcohol and purified by gel excision. The insertion of the Xho1/Spe1 digested Fd fragment and subsequent ligation of the Sac1/Xba1 digested light chain into the pComb3 vector, the transformation into XL1-Blue cells, and the production of phages were performed as described by (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991).

After transformation of the XL1-Blue E.coli cells samples were withdrawn and titrated on plates to determine the library size. These results indicated expression libraries of 7.5x10<sup>6</sup> and 7.7x10<sup>6</sup> cfu (colony forming units) for LD1 and LD2 respectively.

#### c) PCR Primers

VHI 5'-CAC TCC CAG GTG CAG CTG CTC GAG TCT GG-3'

VHII 5'-GTG CTG TCC CAG GTC AAC TTA CTC GAG TCT GG-3'

VHIV 5'-GTC CAG GTG GAG GTG CAG CTG CTC GAG TCT GG-3'

VHV 5'-GTC TGT GCC GAG GTG CAG CTG CTC GAG TCT GG-3'

VHVI 5'-GTC CTG TCA CAG GTA CAG CTG CTC GAG TCA GG-3'

CHI(gl) 5'-AGC ATC ACT AGT ACA AGA TTT GGG CTC-3'

- VL(k) 5'-GT GCG AGA TGT GAG CTC GTG ATG ACC CAG TCT CAA GCT
- CL(k) 5'-T CCT TCT AGA TTA CTA ACA CTC TCC CCT GTT GAA GCT CTT TGT GAC GGG CGA ACT C-3'
- VL(I) 5'C TGC ACA GGG TCC TGG GCC GAG CTC GTG GTG ACT CA-3'
- CL(I) 5'G CAT TCT AGA CTA TTA TGA ACA TTC TGT AGG GGC-3'

### d) Vectors and bacterial strains

The pComb3 vector used for cloning of the Fd and the light chain was obtained from the Scripps Research Institute La Jolla, CA; (Barbas III, C.F. and Lerner, R.A., Companion Methods Enzymol. 2:119, 1991). The *Escherichia coli* strain XL1-Blue used for transformation of the pComb3 vector and the VCSM13 helper phage were purchased from Stratacyte (La Jolla, CA).

### Example 2

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Selection of Rhesus D Phabs from LD1 and LD2 libraries on R1R1 rbc

### a) Absorption and Bio-Panning

A series of three negative absorptions on rbc group O Rh negative were performed for each panning round before positive selection on rbc group O Rh positive (R1R1). Fresh rbc were collected in ACD (acid citrate dextrose) anticoagulant and washed 3 times in 0.9% NaCl. The rbc were counted in Hayems solution and adjusted to  $40x10^6/ml$ . Absorption : 1 ml of phage preparation in PBS/3%BSA was added to rbc group O Rh negative pellet (16x10<sup>6</sup> rbc) in 12 ml tubes (Greiner 187261, Reinach, Switzerland) and incubated at RT for 30 min. with careful shaking. All tubes were pre-blocked in PBS/3% BSA for a minimum of 1hr at RT. The rbc were pelleted by centrifuging for 5 min. 300 x g at 4°C. The resulting phage supernatant was carefully harvested and the process repeated twice more. After the final absorption the phage supernatant was added to the rbc group O Rh positive pellet (16x10 rbc) and again incubated at RT for 30 min. with gentle shaking. Then the rbc were washed at least 5 times in 10 ml ice cold PBS, centrifuged 5 min. 300 x g at 4°C, followed by elution with 200  $\mu l$  of 76 mM citric acid pH 2.8 for 6 min. at R.T. and neutralisation with 200 μl 1M Tris. The rbc were centrifuged 300 x g, 5 min. at 4°C and the resulting supernatant containing the eluted phages was carefully removed and stored with carrier protein

(0.3% BSA) at 4°C ready for re-amplification. The numbers of Rhesus D specific Phabs of each panning round are given in table 5.

Table 5

Selection of Rhesus D+ Phabs from the LD1 and LD2 libraries on R1R1 rbc

	No. of eluted Rhesus D specific phages				
Panning Round No. <sup>a</sup> )	Library D1 cfu	Library D2 cfu			
1	8x10 <sup>6</sup>	4.6x10 <sup>7</sup>			
2	6x10 <sup>7</sup>	1.4x10 <sup>7</sup>			
3	1x10 <sup>8</sup>	7.9x10 <sup>7</sup>			
4	3x10 <sup>8</sup>	1.3x10 <sup>8</sup>			
5	3x10 <sup>8</sup>	1x10 <sup>8</sup>			
6	nd	2.8x10 <sup>8</sup>			

a) For each round 10<sup>12</sup> Phabs were incubated in tubes with rbc Group O Rhesus negative (absorption phase) followed by elution from rbc Group O Rhesus positive (R1R1)

nd = not done

cfu = colony forming units

### Example 3

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## Selection of Rhesus D Phabs from the pooled LD1 and LD2 libraries on DVI+ rbc

a) Absorption on rbc group O Rh negative, phenotypes 1 (r'r, Ccddee) and 2 (ryry, CCddEE)

A series of four negative absorptions on rbc group O Rh negative was performed for each panning round before positive selection on rbc group O Rh DVI positive. The negative absorptions were performed in the following order: Step 1) phenotype 1 treated with bromelase; step 2) phenotype 1 no bromelase; step 3) phenotype 2 treated with bromelase; step 4) phenotype 2

no bromelase. Frozen rbc were thawed into a mixture of sorbit and phosphate buffered saline, left standing in this solution for a minimum of 10 min. and then washed 5 to 6 times in phosphate buffered saline and finally stored in stabilising solution (DiaMed EC-Solution) ready for use. Before panning the rbc were washed 3 times in 0.9% NaCl. followed by counting in Hayems solution. Absorption: 1 ml of phage preparation in PBS/3%BSA was added to an rbc pellet (2x10<sup>8</sup>) as in step 1 in 12 ml tubes (Greiner 187261, Reinach, Switzerland) and incubated at RT for 30 min. with careful shaking. All tubes were pre-blocked in PBS/3% BSA for a minimum of 1hr at RT. The rbc were pelleted by centrifuging for 5 min. 300 x g at 4°C. The resulting phage supernatant was carefully harvested and the process repeated using rbc as detailed above in steps 2, 3, and 4.

## b) Treatment of rbc Rhesus D negative r'r and ryry and Rhesus DVI+ with bromelase

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Bromelase 30 (Baxter, Düdingen, Switzerland) was used to treat rbc Rhesus DVI+ in the same proportions as used in a routine haemagglutination assay, i.e. 10 μl bromelase per 2x10<sup>6</sup> rbc. Thus bromelase was added to the required amount of rbc and incubated at 37°C for 30 min. followed by washing 3 times in 0.9% NaCl, re-counting in Hayems solution and adjusting to the required concentration in PBS/3% BSA ready for Phab panning.

## c) Bio-Panning on bromelase treated Rhesus DVI+ rbc

After the final absorption on rbc ryry non bromelase treated the phage supernatant was divided into 2 equal parts and added either to the enzyme or non enzyme treated rbc group O Rh DVI+ pellet  $(40 \times 10^6)$  respectively and again incubated at RT for 30 min. with gentle shaking. Then the 2 populations of rbc were washed at least 5 times in 10 ml ice cold PBS, centrifuged 5 min.  $300 \times g$  at 4°C, followed by elution with  $200 \mu l$  of 76 mM citric acid pH 2.8 for 6 min. at R.T. and neutralisation with  $200 \mu l$  1M Tris. The rbc were centrifuged  $300 \times g$ , 5 min. at 4°C and the resulting supernatants containing the eluted phages from either the bromelase or non bromelase treated DVI+rbc were carefully removed and stored with carrier protein (0.3% BSA) at 4°C ready for re-amplification. In further rounds of panning the eluted phage from either the bromelase or non bromelase treated DVI+rbc were

kept separate and each followed the absorption protocol steps 1 to 4. The elution step was slightly different compared to panning round 1 as the phage populations were not again divided into 2 parts. Only those phage eluted from bromelase treated DVI+ rbc were also eluted again from bromelase treated DVI+ rbc and only those phage eluted from the non bromelase treated DVI+ rbc were also again eluted from non bromelase treated DVI+ rbc. The numbers of specific Phabs after each panning round are given in table 6.

Table 6 Selection of Rhesus D Phabs from pooled LD1 and LD2 libraries on Rhesus DVI+ red blood cells

	No. of eluted Rhesus DVI+ specific phages				
Panning Round No.a)	- Bromelase cfu	+ Bromelase cfu			
1	1.9x10 <sup>6</sup>	4.4x10 <sup>6</sup>			
2	1.6x10 <sup>6</sup>	4x10 <sup>5</sup>			
3	2.4x10 <sup>7</sup>	4.1x10 <sup>7</sup>			
4	3x10 <sup>6</sup>	5x10 <sup>7</sup>			
5	1×107 <sup>8</sup>	1x10 <sup>8</sup>			
6	nd	3x10 <sup>8</sup>			

a) For each round 10<sup>12</sup> Phabs were incubated in tubes with 2 different phenotypes of rbc Group O Rhesus negative (absorption phase) followed by elution from rbc Group O Rhesus DVI+.

### Example 4

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Monitoring of the panning rounds and determination of the specificity of the enriched Phabs using a rabbit anti-phage antibody

Indirect haemagglutination assay

Freshly collected rbc of different ABO and Rhesus blood groups were washed 3 times in 0.9% NaCl and adjusted to a 3-5% solution (45-  $50x10^7$ /ml) in either 0.9% NaCl or PBS/3% BSA. For each test condition  $50~\mu$ l rbc and 100  $\mu$ l test (precipitated and amplified phage or control antibodies) were incubated together in glass blood grouping tubes (Baxter, Düdingen, Switzerland) for 30 min. at 37°C. The rbc were washed 3 times in 0.9% NaCl

and then incubated with 2 drops of Coombs reagent (Baxter, Düdingen, Switzerland) for positive controls or with 100 µl of 1/1000 diluted rabbit antiphage antibodies (made by immunising rabbits with phage VCSM13 preparation, followed by purification on an Affi-Gel Blue column and absorption on E. coli to remove E. coli-specific antibodies). The tubes were incubated for 20 min at 37°C, centrifuged 1 min at 125xg and rbc examined for agglutination by careful shaking and using a magnifier viewer.

When purified Fab were tested for agglutination, an affinity purified anti-Fab antibody (The Binding Site, Birmingham, U.K.) was used instead of the rabbit anti-phage antibody.

Table 7 shows the results of haemagglutination tests of Phab samples after different panning rounds on R1R1 rbc.

Table 8 shows the results of haemagglutination tests of Phab samples after different panning rounds on Rhesus DVI+ rbc.

Table 9 shows the reactivity pattern of individual Fab clones from libraries LD1 and LD2 with partial D variants.

Table 7 Monitoring of Phabs from LD1 and LD2 libraries by indirect haemagglutination after panning on R1R1 rbc

Phab sample	Library LD1	Library LD2
Panning round	tested on rbo	O Rh D+ (a)
No. 4	+	+
undiluted	+	+/-
1/4	•	•
1/20		
No.5	++	+
undiluted	++	+
1/4	<b>T</b> T	-
1/20	-	
No. 6		+++
undiluted	nd	++
1/4	nd	nd
1/20	nd	iu
Helper phage (b)		
ndiluted, 1/4, 1/20	-	

a ) Indirect haemagglutination was performed in glass tubes using 50  $\mu$ l rbc (40x10 $^7$ /ml) and 100  $\mu$ l Phabs starting at 4x10 $^{11}$ /ml. After 30 min. at 37°C the

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rbc were washed 3 times and further incubated for 20 min. at 37°C with a 1/1000 dilution of rabbit anti-phage antibody.

- b) The M13 helper phage was used as a negative control and showed no non-specific agglutination due to the phage particle alone.
- Agglutination was scored by visual assessment from +++ (strong agglutination) descending to (no agglutination). nd = not done

Table 8 Monitoring of Phabs from pooled LD1 and LD2 libraries by indirect haemagglutination after panning on Rhesus DVI+ rbc

Phab sample Panning round		rbc phenotypes							
	CCDDee	ccddee	Ccddee	CCddEE	DVI (E.J.)	DVI (K.S.)			
Non Bromelase treated rbc DVI+									
Round No.3	a) +++	-	+/-	(+)	+/-	+/-			
Round No. 5	++	·	-	-	•	-			
Bromelase			· -· · · ·						
treated rbc DVI+		-0,	•						
Round No.4	+++	-	+/-	•	. (+)	+/-			
Round No.5	+++	-	+/-	+/-	(+++)	++			
Round No.6	++++	·		'	+++	+++			
LD1 - 6 - 17	reactive with C and E								
LD1/2 - 6 - 3	++++	•	•	•	+/-	nd			
LD1/2 - 6 - 33	++++	-	-	-	+	nd			

a) Agglutination was scored by visual assessment from ++++ (strong agglutination) descending to - (no agglutination). nd = not done

Note: Only those Phabs eluted from bromelase treated DVI+ rbc showed evidence of agglutination against 2 different DVI+ donors.

Table 9

Clonal Analysis of R activity of Fab anti-Rhesus D Clones from Libraries

D1 and LD2 against Partial D Variants

			Parti	al D Vari	iants		
<sup>(a)</sup> Fab Clone No	Rh33	DIII	DIVa	DIVb	DVa	DVI	DVII
LD1 - 40		(b)+++	+	+	+/-	-	++
- 52	_	+++	-	-	+++	-	+++
- 84	_	++	-	-	-	-	+
- 110	(+)	+++	++	+	+	· -	++
- 117	-	+++	<u>-</u> ·	-	-	-	++
LD2 - 1	+++	nd	+++	+++	+	•	+++
- 4	_	+++	•	+	-	•	+
- 5	_	nd	+++	+++	-	-	+++
- 10	(-)	+++	+++	+++	+	-	++
- 11	-	+++	•	-	-	-	++
- 14	+++	+++	+++	+++	+++	-	+++
- 17	-	+++	+++	+	+/-	-	+++
- 20	<b>-</b> ,	+++	+++	-	+/-	-	+++
LD1/2 - 6- 3	++	+++	+++	++	+++	+	++
LD1/2 - 6- 33	+/-	+++	+++	++	+++	+	++

<sup>5</sup> a) soluble Fab preparations were made of each clone followed by indirect haemagglutination.

b) Agglutination was scored by visual assessment from +++ (all cells agglutinated in a clump) descending to - (no cells agglutinated).

### Example 5

## Preparation and purification of Fab antibody fragments for application as diagnostic reagents

After the bio-panning procedures detailed in Examples 2 and 3 a phage population which showed specific agglutination on Rhesus D+ rbc was selected and used to prepare phagemid DNA. More precisely the Phabs selected on R1R1 rbc were used after the 5th and 6th rounds of bio-panning for LD1 and LD2 libraries respectively and after the 5th bio-panning on DVI+ rbc for isolation of the LD1-6-17 clone. In order to produce soluble Fab, the sequence glll coding for the plll tail protein of the phage particle must be deleted.

Phagemid DNA was prepared using a Nucleotrap kit (Machery-Nagel) and the gIII sequence was removed by digesting the so isolated phagemid DNA with Nhe1/Spe1 as described (Burton, D.R., et al., PNAS, 1989). After transformation into XL1-Blue individual clones were selected (nomenclature given in table 1) and grown in LB (Luria Broth) containing 50 μ g/ml carbenicillin at 37°C to an OD of 0.6 at 600 nm. Cultures were induced with 2 mM isopropyl β-D-thiogalactopyranoside (IPTG) (Biofinex, Praroman, Switzerland) and grown overnight at 37°C. The whole culture was spun at 10,000xg for 30 min. at 4°C to pellet the bacteria. The bacterial pellet was treated with a lysozyme/DNase solution to liberate the Fab fragments inside the cells. As some Fab were released into the culture supernatant this was also harvested separately. These Fab preparations were then pooled and precipitated with 60% ammonium sulphate (Merck, Darmstadt, Germany) to concentrate the Fab followed by extensive dialysis in phosphate buffered saline (PBS) and ultracentrifugation at 200,000xg to pellet any insoluble complexes. The Fab preparations were then purified on a ceramic hydroxyapatite column (HTP Econo cartridge, BioRad, Glattbrugg, Switzerland) using a gradient elution of PBS (Buffer A) and PBS + 0.5M NaCl (Buffer B). The linear gradient was programmed to increase from 0-100% Buffer B in 40 min. The Fab was eluted as a single peak between 40-60% Buffer B. The positive fractions as identified by immunodot assay using an anti-Fab peroxidase conjugate (The Binding Site, Birmingham, U.K.) were pooled, concentrated using polyethylene glycol and extensively dialysed

against PBS. The positive fractions from the hydroxyapatite column for each clone were used in a classical indirect haemagglutination assay in glass tubes using either the standard Coombs reagent (Baxter Diagnostics AG Dade, anti-human serum) or an anti-Fab (The Binding Site, Birmingham, U.K.) as the cross linking reagent. These Fab of defined specificity on the Partial D variants as shown on page 18 can be used to type rbc of unknown Partial D phenotype.

### Example 6

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## Construction of complete immunoglobulin genes

The LD2-14 heavy chain V gene (V<sub>H</sub> gene) was amplified from the anti-Rhesus D-Fab-encoding plasmid LD2-14 with the polymerase chain reaction (PCR) using specific primers. The 5'-primer had the sequence: 5'-GGGTCGACGCACAGGTGAAACTGCTCGAGTCTGG-3', whereas the 3'-primer was of the sequence:

5'-GCCGATGTGTAAGGTGACCGTGGTCCCCTTG-3'.

The PCR reaction was performed with Deep Vent DNA Polymerase and the buffer solution (2mM Mg\*\*) from New England Biolabs at the conditions recommended by the manufacturer including 100 pmol of each primer and the four deoxynucleotides at a concentration of 250 μM each. The reaction was run for 30 cycles with the following temperature steps: 60 s at 94°C (extended by 2 min. during the first cycle), 60 s at 57°C and 60 s at 72°C (extended by 10 min. during the last cycle). Post-amplification addition of 3' A-overhangs was accomplished by a subsequent incubation for 10 min at 72°C in the presence of 1 unit Taq DNA Polymerase (Boehringer Mannheim, Germany). The PCR product was purified using the QlAquick PCR purification kit (Qiagen, Switzerland) and cloned into the vector pCRII using Invitrogen's TA cloning kit (San Diego, USA). Having digested the resulting plasmid TAVH14 with *Sal*I and *Bst*EII, the V<sub>H</sub> gene was isolated by preparative agarose gel electrophoresis using Qiagen's QlAquick gel extraction kit.

Vector # 150 (Sandoz Pharma, Basel) which contained an irrelevant but intact human genomic immunoglobulin  $V_{\rm H}$  gene was cut with

Sa/I and BstEII, and the vector fragment was isolated by preparative agarose gel electrophoresis using Qiagen's QIAquick gel extraction kit. Ligation of vector and PCR product was performed at 25°C for 2 hours in a total volume of 20 μl using the rapid DNA Ligation kit (Boehringer Mannheim, Germany). Following ligation, the reaction mix was diluted with 20 μl H<sub>2</sub>0 and extracted with 10 volumes of n-butanol to remove salts. The DNA was then pelleted by centrifugation, vacuum dried and resuspended in 10 μl H<sub>2</sub>0. 5 μl of this DNA solution were electroporated (0.1 cm cuvettes, 1.9 kV, 200 Ω, 25 μFD) with a GenePulser (BioRad, Gaithersburg) into 40 μl of electroporation competent E. coli XL1-blue MRF' (Stratagene, La Jolla), diluted with SOC medium, incubated at 37°C for 1 hour and plated on LB plates containing ampicillin (50 μg/ml). Plasmid-minipreps (Qiagen, Basel) of the resulting colonies were

checked with restriction digests for the presence of the appropriate insert.

With this procedure, the irrelevant resident  $V_H$  gene in vector # 150 was replaced by the amplified anti-Rhesus D  $V_H$  sequence of LD2-14 and yielded plasmid cassVH14. The structure of the resulting immunoglobulin  $V_H$  gene construct was confirmed by sequencing, cut out by digestion with EcoRI and BamHI and gel purified as described above. Expression vector # 10 (Sandoz Pharma, Basel) containing the human genomic immunoglobulin  $C\gamma1$  gene segment was also digested with EcoRI and BamHI, isolated by preparative agarose gel electrophoresis, ligated with the EcoRI / BamHI- $V_H$  gene segment previously obtained from plasmid cassVH14 and electroporated into E. coli XL1-blue MRF' as outlined above. This resulted in a complete anti-Rhesus D heavy chain immunoglobulin gene in the expression vector 14IgG1 (Figure and ).

The LD2-14 light chain V gene ( $V_L$  gene) was amplified from the same anti-Rhesus D-Fab plasmid LD2-14 by PCR using specific primers. The 5'-primer had the sequence:

5'-TACGCGTTGTGACATCGTGATGACCCAGTCTCCAT-3', whereas the 3'-primer was of the sequence:

### 5'-AGTCGCTCAGTTCGTTTGATTTCAAGCTTGGTCC-3'.

PCR reaction, product purification and subsequent cloning steps were analogous to the steps described for the  $V_H$  gene, except that the appropriate light chain vectors were used. Briefly, the  $V_L$  PCR product was

cloned into pCRII vector yielding plasmid TAVL14, excised therefrom with  $\mathit{MluI}$  and  $\mathit{HindIII}$  and isolated by gel extraction. The  $V_L$  gene was subsequently cloned into the  $\mathit{MluI}$  and  $\mathit{HindIII}$  sites of vector # 151 (Sandoz Pharma, Basel) thus replacing the irrelevant resident  $V_L$  gene by the amplified anti-Rhesus D  $V_L$  sequence of LD2-14. Having confirmed the sequence of the resulting plasmid cassVL-14, the  $\mathit{EcoRII}$  Xbal fragment containing the  $V_L$  gene was then subcloned into the restriction sites  $\mathit{EcoRI}$  and Xbal of vector # 98 (Sandoz Pharma, Basel, Switzerland) which contains the human genomic immunoglobulin  $C_K$  gene segment. This procedure replaced the irrelevant resident  $V_L$  gene in plasmid # 98 and yielded the expression vector 14kappa which contains the complete anti-Rhesus D light chain immunoglobulin gene.

The mouse myeloma cell line SP2/0-Ag 14 (ATCC CRL 1581) was cotransfected by electroporation with the expression vectors 14lgG1 and 14kappa previously linearized at the unique EcoRI and NotI cleavage site, respectively. The electroporation was performed as follows: exponentially growing cells were washed twice and suspended in phosphate buffered sucrose (272 mM sucrose, 1 mM MgCl<sub>2</sub>, 7 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 7.4) at a density of 2 x 10<sup>7</sup> cells/ml. 0.8 ml of cells were added to a 0.4 cm cuvette, mixed with  $15~\mu g$  of linearized plasmids 14lgG1 and 14kappa, held on ice for 15 min., electroporated with 290 Volts, 200  $\Omega$ , 25  $\mu FD$ , put back on ice for 15 min., transferred to a T75 cell culture flask with 20 ml of cold RPMI 1640 medium (10% heat inactivated fetal bovine serum, 50 μM beta-mercaptoethanol), left for 2 h at room temperature and then incubated for 60 h at 37°C. After this period, the cells were transferred to 50 ml of medium containing 1 mg/ml G418 for selection. Stable transfectants were then selected in the presence of increasing concentrations of methotrexate to amplify the integrated DNA and thus increasing the expression of the corresponding antibody rD2-14.

Expression of rD2-14 in the culture's supernatant (SrD2-14) was monitored by an enzyme linked immuno-sorbent assay (ELISA) specific for human  $\gamma 1$  and kappa chains. Quantification of the Rhesus D specific immunoglobulins in the anti-D assay according to Ph. Eur. revealed between 1.1 and 11.4  $\mu$ g/ml of agglutinating antibody in such supernatants. They tested agglutination negative for Rhesus negative rbc and revealed the same agglutination potential against partial D variants as the Fab LD2-14 expressed in E. coli. The data are shown in table 10.

Table 10

Comparative analysis of reactivity of Fab anti-Rhesus D clone LD2-14

and antibody rD2-14 against partial D variants

				Partial D Variants					
	R1R1	ιτ ·	Rh33	DIII	DIVa	DIVb	DVa	DVI	DVII
LD2-14	+++	_	+++	+++	+++	+++	+++	-	+++
SrD2-14	+++	-	+++	+++	+++	+++	+++	-	+++
ТСВ	-	-		_					0

Agglutination was scored by visual assessement from +++ (all cells agglutinated in a clump) descending to - (no cells agglutinated).

LD2-14: Fab fragment prepared as described in Example 5;

SrD2-14: cell culture supernatant containing antibody rD2-14;

TCB: cell culture supernatant of untransfected cells.

### **Claims**

1. Polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include Rhesus D-specific CDR 1, CDR 2 and CDR 3 regions of pairs of amino acid sequences  $V_{\rm H}$  and  $V_{\rm L}$  with the same or different identification numbers according to the figures given in the table below:

<del></del>		V	'н			,	V <sub>L</sub>	
Identi- fication No.	Figure	CDR 1 base pair No.	CDR 2	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.
LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288
LD1-84	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285
	Fig. 4a	91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285
LD1-110	<del>-</del>	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288
LD1-117	Fig. 5a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294
LD2-I	Fig. 6a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282
LD2-4	Fig. 7a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288
LD2-5	Fig. 8a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294
LD2-10	Fig. 9a	<del>                                     </del>	148-198	295-342	Fig. 10b	64-96	142-162	259-285
LD2-11	Fig. 10a	91-105	148-198	295-342	Fig. 11b		142-162	259-285
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 12b		142-162	259-285
LD2-17	Fig. 12a	91-105		295-342	Fig. 13b	<del> </del>	142-162	259-285
LD2-20	Fig. 13a	91-105	148-198		Fig. 14b		142-162	259-285
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 15b		142-162	259-285
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342		-	142-162	259-285
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	04-30	1172-102	1

- 2. Polypeptides according to claim 1 which include Rhesus D-specific CDR 1, CDR 2 and CDR 3 regions of pairs of amino acid sequences  $V_H$  and  $V_L$  with the same identification numbers according to the figures given in the table of claim 1.
- 3. Polypeptides according to claim 1 which include regions with the amino acid sequences  $V_H$  and  $V_L$  and have identification numbers according to the figures given in the table of claim 1.

- 4. Polypeptides according to claim 1, 2 or 3 characterised as antigen binding Fab fragments.
- Polypeptides according to claim 1, 2 or 3 comprising immunoglobulin heavy and light chains capable of forming complete anti-Rhesus D antibodies.
- 6. DNA sequences coding for polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include regions with the Rhesus D-specific CDR 1, CDR 2 and CDR 3 segments of pairs of DNA sequences V<sub>H</sub> and V<sub>L</sub> with the same or different identification numbers according to the figures given in the table below and functional equivalents thereof:

		\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	/н	٧ <sub>L</sub>							
Identi- fication No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.			
LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288			
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288			
LD1-84	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285			
LD1-110	Fig. 4a	91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285			
LD1-117	Fig. 5a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288			
LD2-1	Fig. 6a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294			
LD2-4	Fig: 7a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282			
LD2-5	Fig. 8a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288			
LD2-10	Fig. 9a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294			
LD2-11	Fig. 10a	91-105	148-198	295-342	Fig. 10b	64-96	142-162	259-285			
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	64-96	142-162	259-285			
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b	64-96	142-162	259-285			
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b	64-96	142-162	259-285			
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b	64-96	142-162	259-285			
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b	64-96	142-162	259-285			
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285			

7. DNA sequences according to claim 6 coding for polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens which include regions with the Rhesus D-specific CDR 1, CDR 2 and CDR 3 segments of pairs of DNA sequences V<sub>H</sub> and V<sub>L</sub> with the same

identification numbers according to the figures given in claim 6, and functional equivalents thereof.

- 8. DNA sequences according to claim 6 or 7 which include regions with the DNA sequences  $V_{\text{H}}$  and  $V_{\text{L}}$  with the identification numbers according to the figures given in claim 6.
  - DNA sequences according to claim 6, 7 or 8 coding for polypeptides capable of forming antigen binding Fab fragments.
  - 10. DNA sequences according to claim 6, 7 or 8 coding for polypeptides capable of forming complete anti-Rhesus D antibodies.
- 11. A process for preparing recombinant polypeptides capable of forming antigen binding structures, e.g. Fab fragments, with specificity for Rhesus D antigens which process comprises the following steps in sequential order:
  - a) boosting of an individual capable of forming anti-Rhesus D antibodies with Rhesus D positive red blood cells,
  - b) isolating mononuclear cells from the individual,

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- c) isolating total RNA from the mononuclear cells,
- d) preparing a cDNA by using an oligo(dT)primer and reverse transcribing of the mRNA with M-MuLV reverse transcriptase and amplifying the cDNA repertoire by a polymerase chain reaction using immunoglobulin gene family specific primers,
- e) creating a phage display library by inserting the DNA coding for the heavy and light chain of the Fab polypeptide into a phagemid vector; the DNA for the heavy chain is inserted in frame to the gene coding for the phage protein pIII which allows the expression of a Fab pIII fusion protein on the surface of the phage,
- f) transforming bacterial cells with the obtained recombinant plasmids, cultivating of the transformed bacterial cells and co-expression of the heavy and the light chain of a Fab on filamentous phage particles,

- g) amplifying the Fab-carrying phage in bacteria,
- h) selecting individual phage clones by several rounds of panning on Rhesus positive red blood cells.
- i) isolating the plasmid DNA from the selected clones and cutting out the gIII gene,
- j) transforming bacterial cells with the obtained plasmid, cultivating of the transformed bacterial cells expressing the Fab, and isolating the Fab fragments.
- 12. A process for selecting recombinant polypeptides capable of forming antigen binding structures with specificity for Rhesus D antigens and in particular showing reactivity with the Partial Rhesus DVI Variant and without any evidence of reactivity with red blood cells of Rhesus negative phenotypes in particular without reactivity against the Rhesus alleles C, c, E, and e which process comprises the following steps in sequential order:
- a) performing several negative absorptions on the following red blood cells: phenotype 1 (r'r, Ccddee) treated with bromelase, phenotype 1 not treated with bromelase, phenotype 2 (ryry, CCddEE) treated with bromelase and phenotype 2 not treated with bromelase,
- b) performing a positive absorption on DVI+ red blood cells with or without bromelase treatment,
  - c) determining the titer of phage binding to DVI+ red blood cells
  - d) repeating steps a), b) and c) until the titer of phage binding to DVI+ red blood cells has reached a satisfactory level.

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- 13. A process according to claim 12, wherein the recombinant polypeptides capable of forming antigen binding structures are Fab fragments.
- 14. Anti-Rhesus D antibodies having heavy and light chain variable
   regions comprising the Rhesus D-specific CDR 1, CDR 2 and CDR 3

sequences of pairs of amino acid sequences  $V_{\text{H}}$  and  $V_{\text{L}}$  having the same or different identification numbers according to the table below:

		\	/н		V <sub>L</sub>						
Identi- fication No.	Figure	CDR 1 base pair No.	CDR 2	CDR 3 base pair No.	Figure	CDR 1 base pair No.	CDR 2 base pair No.	CDR 3 base pair No.			
LD1-40	Fig. la	91-105	148-198	295-342	Fig. 1b	64-96	142-162	259-288			
LD1-52	Fig. 2a	91-105	148-198	295-342	Fig. 2b	64-96	142-162	259-288			
LD1-84	Fig. 3a	91-105	148-198	295-342	Fig. 3b	64-96	142-162	259-285			
LD1-110	Fig. 4a	91-105	148-198	295-342	Fig. 4b	64-96	142-162	259-285			
LD1-117	Fig. 5a	91-105	148-198	295-345	Fig. 5b	64-96	142-162	259-288			
LD2-I	Fig. 6a	91-105	148-198	295-342	Fig. 6b	61-99	145-165	262-294			
LD2-4	Fig. 7a	91-105	148-198	295-342	Fig. 7b	64-96	142-162	259-282			
LD2-5	Fig. 8a	91-105	148-198	295-342	Fig. 8b	64-96	142-162	259-288			
LD2-10	Fig. 9a	91-105	148-198	298-345	Fig. 9b	61-102	148-168	265-294			
LD2-11	Fig. 10a	91-105	148-198	295-342	Fig. 10b	64-96	142-162	259-285			
LD2-14	Fig. 11a	91-105	148-198	295-342	Fig. 11b	64-96	142-162	259-285			
LD2-17	Fig. 12a	91-105	148-198	295-342	Fig. 12b	64-96	142-162	259-285			
LD2-20	Fig. 13a	91-105	148-198	295-342	Fig. 13b	64-96	142-162	259-285			
LD1-6-17	Fig. 14a	91-105	148-198	295-351	Fig. 14b	64-96	142-162	259-285			
LD1/2-6-3	Fig. 15a	91-105	148-198	295-342	Fig. 15b	64-96	142-162	259-285			
LD1/2-6-33	Fig. 16a	91-105	148-198	295-342	Fig. 16b	64-96	142-162	259-285			

- 15. Anti-Rhesus D antibodies having heavy and light chain variable regions comprising the Rhesus D-specific CDR 1, CDR 2 and CDR 3
   sequences of pairs of amino acid sequences V<sub>H</sub> and V<sub>L</sub> having the same identification numbers as indicated in the table of claim 14.
  - 16. Anti-Rhesus D antibodies according to claim 14 or 15 which include pairs of amino acid sequences  $V_{\rm H}$  and  $V_{\rm L}$  having the identification numbers according to the figures, as indicated in the table of claim 14.
  - 17. Anti-Rhesus D antibodies according to claims 14, 15, or 16 wherein the immunoglobulin constant regions are of at least one of the defined isotypes IgG1, IgG2, IgG3 or IgG4.

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- 18. A process for preparing complete anti-Rhesus D antibodies according to one of the claims 14 to 17, comprising in sequential order the steps of
  - a) amplifying separately the members of a pair of a heavy chain V gene segment and a light chain V gene segment containing Rhesus Dspecific CDR 1, CDR 2 and CDR 3 regions as depicted in Figs. 1a -16a and 1b - 16b, respectively, from an anti-Rhesus D-Fab-encoding plasmid by carrying out a polymerase chain reaction with specific primers,
- b) preparing separately the genes of a complete anti-Rhesus D immunoglobulin heavy chain and a complete anti-Rhesus D immunoglobulin light chain in suitable plasmids containing the immunoglobulin constant region gene segments coding for either one of the human γ1, γ2, γ3 and γ4 heavy chains and for the human κ or λ light chain and transforming the obtained plasmids separately in suitable E. coli bacteria, and
  - c) cotransfecting the obtained plasmids into suitable eukaryotic host cells, cultivating of the cells, separating the non-transformed cells, cloning of the cultures, selecting the best producing clone, using it as a production culture and isolating the complete antibodies from the supernatant of the cell culture.
  - 19. A pharmaceutical composition comprising at least one polypeptide according to the definition of claim 1, 2 or 3 or at least one anti-Rhesus D antibody according to one of the claims 14 to 17 for the prophylaxis of haemolytic disease of the newborn, for the treatment of idiopathic thrombocytopenic purpura and mistransfusions of Rhesus incompatible blood.
    - 20. A diagnostic composition for Rhesus D typing comprising Fab fragments according to claim 4 or anti-Rhesus D antibodies according to one of the claims 14 to 17.

## Fig. 1a

## LD1-40-VH sequence

		9			18			27			36			45			
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCT	GGG	AGG	TCC	C
																	-
Q	V	К	L	L	Е	S	G	G	G	V	V	Q	P	G	R	·S	
		63			72									99			1
AGA	CTC	TCC	TGT	ATA	GCG	TCT	GGA	TTC	ACC	CTC	AGG	AAT	TAT	GCC	ATG	CAC	7
 R	L	s			A	s	G	F	T	L	R	N	Y	A	М	Н	
												←		- CD	RI —	<del></del>	
		117			126			135			144			153			1
GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGG	CTG	GAG	TGG	GTG	GCA	GGT	ATA	TGG	TTT	G
v	 R	0		 P					E	W	· v	Α	G	I	W	F	
•	•	-											←—		CDR2		
		171			180			189	٠.		198			207			
GGA	AGT	AAC	AAA	AAC	TAT	GCA	GAC	TCC	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	P
G	s	N	ĸ	N	Y	A	D	 ș	V	К	Ġ	R	F	T	I	S	
					- CDF	₹2					<del>&gt;</del>						_
		225			234			243			252		~~~			C	
GAC	AAT	TCC	AAG	AAC	ACG	CTG	TAT	CTG	CAA	CTG	AAC	AGC	CTG	AGA	GAC	GAG	-
D	N	s	К	N	T	L	Y	Ĺ	Q	L	N	s	L	R	Đ	Ε	
		279			288			297			306			315			3
ACG	GCT	GTG	TAT	TAT	TGT	GCG	AGA	GAG	CGA	GCA	GCA	CGT	GGT	ATT	TCT	AGG	T
 Т	 A						R	 E	R	`	Α	R	G	I	s	R	_
-		-	_	-				4				- CD	R3				
								351			360			369			
		333			342												
TAT	TAC	333 TAC			GTC	TGG	GGC	AAA	GGG	ACC	ACG	GTC	ACC	GTC	TCC	CCA	
	TAC	TAC	ATG	GAC	GTC V	TGG 	GGC	AAA  K	GGG 	ACC T	ACG T	GTC  V	ACC T	GTC	TCC  S		

## Fig. 1b

## LD1-40-VL sequence

63 TGC C  C	Q CGG  R	S GCA  A	CCA P 72 AGT S	TCC S S CAG	S AGC	CTG L 81 ATT	TCT  S	GCA A	TCT  S 90	v	G	D 99	R	v	T 108
63 TGC C  C	CGG  R ←	GCA  A	72 AGT  S	CAG	AGC	81 ATT			90			99			108
rgc c  c 117	CGG  R ←	GCA  A	AGT  S	CAG  Q	AGC	ATT	AGG	AGC	90 CAT	mm.c		99			
 c 117	R ←	A	 s	 Q			AGG	AGC	$C\Delta T$						
117	<del></del>	<u> </u>			s					TTG	AAT	TGG	TAT	CAG	CAG
117	<del></del>	<u> </u>				I	R	S	Н	L	N	W	Y	Q	Q
117			126			CDR	l —				<del>&gt;</del>				
CCC A	AAA	CCC				135			144			153			162
GGG A			CCT	AAG	TTG	CTG	ATC	TAT	GGT	GCG	TCC	ACT	TTG	CAA	AGT
 G				 V		- <b></b>	т				S	т	L	0	 s
G	V	A	E	K	u		_	•				CDR2			
171			180			189									216
CCA T	TCA	AGG	TTC	AGT	GGC	AGT	GGC	TCT				TTC	ACT	CTC	ACC
								, <i>-</i>							
P	S	R	F	S	G	S	G	S	G	A	V	F	T	L	T
225			234			243			252			261			270
AGT C	CTA	CAA	CCT	GAA	GAT	TTT	GCA	ACT	TAC	TAC	TGT	CAA	GAG	AGT	TAC
	L	Q	P	E	.D	F	Α	T	Y	Y	C	Q	E	s	Y
s			200			207			306			315			
		ATC	ACC	TTC	GGC	CAA	GGG	ACA	CGA	CTG	GAG		AAA	3'	
279	CTA		 Т	 F	 G	Q	 G		R	L	E	T	ĸ		
- :	79	79 CT CTA	CT CTA ATC	CT CTA ATC ACC	CT CTA ATC ACC TTC	CT CTA ATC ACC TTC GGC	CT CTA ATC ACC TTC GGC CAA	CT CTA ATC ACC TTC GGC CAA GGG	CT CTA ATC ACC TTC GGC CAA GGG ACA	CT CTA ATC ACC TTC GGC CAA GGG ACA CGA	CT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG	CT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG	CT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT	CT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT AAA	CT CTA ATC ACC TTC GGC CAA GGG ACA CGA CTG GAG ACT AAA 3'

## Fig. 2a

## LD1-52-VH sequence

		9			18			27			36			45			54
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CT
Q	v	к	L	L	E ·	s	G	G	G	v	v	Q	P	G	G	S	. T
		63			72						90			99			10
AGA	CTC	TCC	TGT	GAA	GCG	TCT	GGA	TTC	GCC	CTC	AGA	AGT	TCT	GGC	ATG	CAC	TG
 R		 S		 E	 A	 S	 G	 F	 A		 R	s	s	G	M	Н	W
	1,		Ŭ	_								<del></del>	Tari	CDR1		<del></del>	
GTC	CGC	117 CAG	GCT	CCT	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG	GCA	СТТ	153 ATA		TTT	16 GA
	 R	 Q					 G					A	L	I	W	F	D
								100			198			207	CDR2		21
GGA	AGT	171 ATC	AGA	TCG	TAT	GCA	gaa	TCC	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	
 G	 S		 R	 S	 Y	Α	E	 s	v	<b>-</b>	G	R	F	T	I	\$	P
					- CDF	R2					<del></del>						
GAC	ACT	225 TCC	AAG	AAC	234 ACC	CTA	TAT	243 CTC	CAA	ATG	252 CGC	AGT	CTG	261 AGT	GCC	GAC	GA
 D		 S	 К	N		L	Y	L L	Q	M	R	s	L	s	A	D	Γ
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGA	297 GAC	aag	GCG	306 GTT	CGG	GGA	315 ATT	AGC	AGG	32 TA
 T	 A	v	Y	Y	c	Α	R		К	Α		R		_	s	R	Y
								<del></del>				(		369			•
AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	AAA	GGG	ACC				GTC	TCC	TCA	3
N	Y	Y CDR3	М	D	v	₩ →	G	К	G	T	Т	V	T	v	5	s	

## Fig. 2b

#### LD1-52-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
 V		 T	Q	 S	P	s	s	L	s	Α	s	v	G	D	R	V	T
ATC	ACT	63 TGC	CGG	GCA	72 AGT	CAG	AAC	81 ATT	ATC	CGC	90 TAT		AAT	99 TGG	TAT	CAG	108 CAG
 I			 R	 A	 S	Q	N	I.	I	R	Y	L	N	W	Y	Q	Q
_	_		4					CDR	1				<del></del>	•			
		117			126			135			144			153			162
AAG	CCA	GGG	AAA	GCC	CCT	AGG	CTC	CTG	ATC	TAT	GGT	GCG	TCC	ACT	TTG	CAA	AGT
<b>-</b>	 P													T	L	Q	s
											+				R2 —		
		171			180			189		•							216
GGG	GTC	CCY	TCA	AGG	TTC	AGT	GGC	AGT	GGA	TCT	GGĢ	ACA	GAT	TTC	ACT	CIC	ACC
 G		 Р	 S	 P.	F	5	G	s.	G	s	G	T	D	F	T	L	T
		225			234			243			252			261			270
ATC	AGT	AGT	CTG	CAA	CCT	GAA	GAT	TTT	GCA	ACT	TAC	TAC	TGT	CAA	CAG	AGT	TAG
				 Q								Y		Q	Q	s	Y
		279			288			297			306			315			
CGT	ACC	CCT	CCA	TTC	ACT	TTC	GGC	CCT	GGG	ACC	AAA	GTG	GAG	ATC	AAA	3,	
								 P.					E				
R	T	P	₽	F	T		G	₹.	G			•	_	_			

## Fig. 3a

#### LD1-84-VH sequence

CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CTC  Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GAA GCG TCT GGA TTC ACC CTC AGA AGT TCT GGC ATG CAC TG  R L S C E A S G F T L R S S G M H W  GTC CGC CAG GCT CCT GGC AAG GGG CTG GAG TGG GTG GCA CTT ATA TGG TTT GAC  V R Q A P G K G L E W V A L I W F D  GGA AGT ATC AGA TCG TAT GCA GAA TCC GTG AAG GGC CGA TTC ACC ATC TCC AG  G S I R S Y A E S V K G R F T I S R  CDR2  GAC ACT TCC AAG AAC ACC CTA TAT CTC CAA ATG CGC AGT CTG AGT GCC GAC GAC  D T S K N T L Y L Q M R S L S A D D  ACG GCT GTG TAT TAC TGT GCG AGA GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG GAC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG AAG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG TACC AGG AGG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC AGG AGG GAC AAG GAC AAG GAC AAG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC ACC AGG AGG AGC AAG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC ACC AGG AGG AGC AAG GAC AAG GCG GTT CGG GGA ATT AGC AGG TACC ACC ACC ACC ACC ACC ACC ACC ACC AC			9			18			27			36			45			54
63	CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTG
AGA CTC TCC TGT GAA GCG TCT GGA TTC ACC CTC AGA AGT TCT GGC ATG CAC TG  R L S C E A S G F T L R S S G M H W  117	 Q		 К		L	 E	 s	G	G	G	v	v	Q	P	G	G	s	L
R L S C E A S G F T L R S S G M H W    117	AGA	стс	63 TCC	TGT	GAA	72 GCG	тст	GGA	81 TTC	ACC	СТС	90 AGA	AGT	TCT		ATG	CAC	108 TGG
THE STANDARD													s	s	G	М	н	W
V       R       Q       A       P       G       K       G       L       E       W       V       A       CDR2       —       CDR2       —       —       CDR2       —       207       21       —       207       21       —       208       —       ACC       AG       AG       ACC       AG       ACC       AG       ACC       ACC       ACG       ACG       ACG       CTA       TAT       CTC       CAA       ATG       CGC       AGT       CTG       AGT       GCC       GAC       GAC       GAC       GAC       AGG       GAC       AGG       AGG       GAC       AGG       GAC       AGG       AGG       AGG       GAC       AGG       GAC       AGG       GAC       AGG       AGG       AGG       ATT       AGC       AGG       AAG       AGC       AGG       AAG       AGG       AGG       AAG       AGG       AAG       AGG       AAG       AAG </td <td>GTC</td> <td>CGC</td> <td>117 CAG</td> <td>GCT</td> <td>CCT</td> <td>126 GGC</td> <td>AAG</td> <td>GGG</td> <td>135 CTG</td> <td>GAG</td> <td>TGG</td> <td>144 GTG</td> <td>•</td> <td></td> <td>153</td> <td></td> <td></td> <td>162 GAT</td>	GTC	CGC	117 CAG	GCT	CCT	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG	•		153			162 GAT
GGA AGT ATC AGA TCG TAT GCA GAA TCC GTG AAG GGC CGA TTC ACC ATC TCC AGG S I R S Y A E S V K G R F T I S R  CDR2  225  CDR2  234  CAC ACT TCC AAG AAC ACC CTA TAT CTC CAA ATG CGC AGT CTG AGT GCC GAC GAC GAC GAC GAC GAC GAC GAC GAC	v	 R	Q	Α	P		ĸ	G	L	E	w	v	A				-	D
CDR2  225 234 243 252 261 276  GAC ACT TCC AAG AAC ACC CTA TAT CTC CAA ATG CGC AGT CTG AGT GCC GAC GAC  D T S K N T L Y L Q M R S L S A D D  ACG GCT GTG TAT TAC TGT GCG AGA GAC GAG GGG GTT CGG GGA ATT AGC AGG TA  T A V Y Y C A R D K A V R G I S R Y  AAC TAT TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC TCC TCA 3  AAC TAT TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC TCC TCA 3	GGA	AGT	171 ATC	AGA		TAT	GCA'	GAA	TCC	GTG	AAG	GGC	CGA	TTC	207 ACC	ATC	TCC	216 AGA
225 GAC ACT TCC AAG AAC ACC CTA TAT CTC CAA ATG CGC AGT CTG AGT GCC GAC GA  D T S K N T L Y L Q M R S L S A D D  279 ACG GCT GTG TAT TAC TGT GCG AGA GAC AAG GCG GTT CGG GGA ATT AGC AGG TA  T A V Y Y C A R D K A V R G I S R Y  AAC TAT TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC TCC TCA 3  AAC TAT TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC TCC TCA 3	<b>G</b>	s	I								ĸ	G	R	F	T	I	S	R
D T S K N T L Y L Q M R S L S A D D  279	GAC	ACT	225 TCC			224			243 CTC	CAA	ATG	252 CGC	AGT	CTG	261 AGT	GCC	GAC	270 GAC
279 ACG GCT GTG TAT TAC TGT GCG AGA GAC AAG GCG GTT CGG GGA ATT AGC AGG TA  T A V Y Y C A R D K A V R G I S R Y  333 342 351 360 369 AAC TAT TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC ACC GTC TCA 3	D		s	к	N		L	Y			М	R	S	L	s	A	D	Ď
T A V Y Y C A R D K A V T C DR3  333 342 351 360 369  AAC TAT TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC ACC GTC TCA 3	ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGA	297 GAC	AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGC	AGG	324 TAC
333 342 351 360 369  AAC TAT TAC ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC ACC GTC TCA 3	T	A	v	Y	Y	С	A	R	D	ĸ					_		R	Y
N Y Y M D V W G K G T T V T V S S	AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351			360			369		TCA	3'
CDR3	 N	Y	_			v	W	G	к	G	T	T	v	T	v	S	S	

## Fig. 3b

#### LD1-84-VL sequence

		9			18						36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	ATA	GGA	GAC	AGA	GTC	ACC
v		T	Q	 s	P	 s	s	L	s	Α	s	I	G	D	R	v	Т
		63			72			81			90	<b>m</b> mc	አክሞ	99	ጥአጥ	CNG	108
ATC	ACC	TGC	CGG	GCA	AGT	CAG	AGT	ATC	ATC	AGG	171			TGG			
I	T	С	R	А	s	Q	S	I	I	R	Y	L	N	W	Y	Q	1
			←—					CE	)R1 -								
		117			126			135			144			153			16
AAA	CCA	GGA	AAA	GCC	CCT	AAA	CTC	CTC	ATC	TTT	GCT	GCA	TCG	AAT	TTG	CAA	AC
к	P	 G	к	A	P	ĸ	L	L		F	A	A	S	N	L	Q	T
											<del></del>			CDR2 207			21
GGG	GTC	171 CCA	TCC	AGG	180 TTC	AGT	GGC	189 AGT	GGA	TCT	198 GGG		GAT	TTC	ACT	CTC	
																	т
G	V	P	S	R	F	S	G.	S	G	S	G	T	D	F	T	L	1
	٠	225			234			243			252			261			27
ATC	AGT	GAC	CTG	CAG	CCT	GAG	GAT	TTC	GCA	ACT	TAC	TAC	TGT	CAA	CAG	AGT	TA
 I	 s	D	L	Q	P	E	D	F	A	T	Y	Y	С	Q	Q	S	Y
		279			288			297			306			315			
AGT	AGG	CCG	TTC	ACT	TTT	GGC	CGG	GGG	ACC	AGC	CTG	GAC	ATC	AAA	3'		
 s	 R	 P	 F	т	 F	<b>-</b>	 R	 G	T	s	L	D	I	К			
	(	DR3															

### Fig. 4a

#### LD1-110-VH sequence

		9			18			27			36			45			5
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCT	GGG	AGG	TCC	CT
0	 V	 K			 E		G			v	v	Q	P	G	R <sub>.</sub>	s	L
~		63	TGT		72	ma <b>m</b>	cca	81	N.C.C	ርሞር	90	דממ	<b>ጥ</b> ልጥ	99 GCC	ATG	CAC	10 TG
AGA	CTC	TCC	TGT	ATA	GCG	TCT	GGA		ACC								
 R	L	s	С	I	Α	s	G	F	T	L	R	N		Α	M	Н	W
		117	GCT	CCA	126	פממ	GGG	135 CTG	GAG	ТGG	144 GTG	← GCA		153		TTT	16
GTC		CAG											-				_
v	R	Q	Α	P	G	ĸ	G	L	E	W	V			I		F	Ι
								100			198		<del></del>	207	- CDI	٠ <u>-</u>	21
GGA	AGC	17:1 AAC	AAA	AAC	TAT	GCA	GAC	TCC	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	A
	 s			N		A	D	s	v	K	G	R	F	T	I	s	Ī
											<del></del>	•					٠,
		225			224			213			252		om c	261	ccc	CNC	2
GAC	AAC	TCC	AAG	AAC	ACT	CTG	TTT	CTG	CAC	ATG	AAC	AGC					
 D		 s		N	т	L	F	L	Н	M	N	s	L	R	A	E	1
					200	,		297			306			315			3
ACG	GCT	279 ACA	TAT	TAC	TGT	GCG	AGA	GAG	AGG	GCG	ATT	CGG	GGA	ATC	AGT	AGA	T
													 G				-,
T	Α	T	Y	Y	С	Α	R	E	R	A		<del></del> (		_	_		
		222			342		•	351			360			369			
AAT	TAC	333 TAC	ATG	GAC	GTC	TGG	GGC	AAG	GGG	ACC	ACG	GTC	ACC	GTC	TCC	TCA	
N	Y	Y	М	D	v	W	G	к	G	T	T	V	T	٧	s	S	

### Fig. 4b

#### LD1-110-VL sequence

		9			18			27			36			45			54	
GT	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	
v	М	T	Q	s	P	s	s	L	s	A	s	v	G	D	R	v	T	
		63			72			81			90			99				
ATO	ACT	TGC	CGG	GCA	AGT	CAG	AGC	ATT	CGA	AGC	TCT	TTA	AAT	TGG	TAT	CAG	CAG	
I	т	С	R	A	s	Q	s	I	R	s	s	L	N	W	Y	Q	Q	
			<del></del>					CDR	L				<del></del>					
		117			126			135			144							
AA	A CCA	GGG	AAA	GCC	CCT	AAA	GTC	CTG	ATC	TAT	GCT	GCA	TCC	AGT	TTG	CAA	AGT	
ĸ	P	G	ĸ	A	P	<b>-</b>		L	I	Y							s	
														- CD				
		171			180			189			198			207	. cm	cmc	216	
GG	GTC	CCA	TCC	AGG	TTC	AGT	GGC	AGA	GGA	TCT	GGG	ACA	GAT		ACI			
G	v	P	s	R	F	S	G	R	G	S	G	T	D	F	T	L	T	
		225			234			243			252			261			270	
AT	AGC	AGT	CTG	CAG	CCT	GAA	GAT	TTT	GCG	ACT	TAT	TAT	TGT	CAA	CAG	AGT	TCC	
I	s	s	L	Q	P	E	D	F	A	Т	Y	Y	С	Q	Q	s	s	
		270			288			297			306			315				
AG'	r TCC	TCG	TGG	ACG	TTC	GGC	CAA	GGG	ACC	AAG	GTG	GAA	ATC		3'			
s	 s	s	W	Ţ	F	G	Q	G	т	K	v	Ε	I	K				
	— сі	DR3 -		<del></del>														

### Fig. 5a

#### LD1-117-VH sequence

		9	CTG	ama.	18	mc »	CCA	27	ccc	CTC.	36 GTC	CAG	CCT	45 GGG	AAG	тсс	54 CTG
CAG	GTG	AAA	CTG	CTC	GAG	TCA	GGA	GGA									
Q	v	К	L	L	Е	s	G	G	G	V	V	Q	P	G	K	S	L
		63			72			81			90						108
AGA	CTT	TCC	TGT	GCA	GCG	TCT	GGA	TTC	AGT	TTC	TAA	AGC	CAT	GGC	ATG	CAC	TGG
 R		 S	c		Α	s	G	F	s	F	N	s					W
												<del></del>		CDR1		<del></del>	
		117			126			135			144			153			162
GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGG	CTG	GAG	TGG	GTG	GÇA	TTT	ATA	TGG	TTT	GAT
																	D
V	R	Q	Α	5	G	K	G	L	E	W	٧	А	r	-	~~~	-	_
														207	CDRZ		216
		171			180			189			198	CCA			አጥሮ	ACC	
GGC	AGT	AAT	AAA	TAC	TAT	GCA	GAC	TCC	GTG	AAG	GGC	CGA	110	ACC	A1C		
	 S	 N	к	Y	Υ		D	s	v	ĸ	G	R	F	T	I	T	R
					— ci	DR2 -					<del></del>						
		225			234			243			252						270
GAC	AAC	TCC	AAG	AAC	ACG	CTG	TAT	CTG	CAA	ATG	AAC	AGC	CTG	AGA	GCC	GAG	GAC
			 К											R			D
														215			324
		279			288			297			300	N.C.C	CTA				
ACG	GCT	GTC	TAT	TAC	TGT	GCG	AGA	GAG	ACC	TCA	GIA	AGG					
т	 A	v	Υ	Υ	c	A	R	E	T	s	V	R	L	G	Y	S	R
												- CD	R3 —				270
		333			342			351			360			369			378 TCA
TAC	AAT	TAC	TAC	ATG	GAC	GTC	TGG	GGC	AAA	GGG	ACC	ACG	GTC	ACC	AIC	106	TCA
 Y	 N	 Y	 Y	м		v	w	G	ĸ	G	T	T	٧	Ť	I	s	s
_																	

## Fig. 5b

## LD1-117-VL sequence

			9			18						36			45			5
GT	TA 5	3 .	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	AC
v	M	-	T	Q	s	P	s	s	L	s	A	s	V	G	D	R	v	T
			63			72			81			90			99		~. ~	10
ATO	AC.	r	TGC	CGG	GCA	AGT	CAG	AGC	ATT	AGG	AGC	CAT	TTG	AAT	TGG	TAT	CAG	
· I	- <b></b> -	•	С.	R	A	S	Q	s	I	R	S	H	L	N	W	Y	Q	Ç
									CE					<del></del>				•
			117			126			135						153		a	16
AA	CC	4	GGG	AAA	GCC	CCT	AAG	CTC	CTG	ATC	TAT	GCT	GCA	TCC	AGT	TTG	CAA	G
		-											۸			L	0	_
K	P		G	К	Α	P	K	r	L	÷		,	. A		CDR2		<u> </u>	
						100			100			198			207			21
	~ ~~	~	171	TIC N	AGG	TRO	ACT		TOD	GGA	тст			GAT			CTC	
66		_	CCA	ICA			701											
G	ν		P	S	R	F	s	G	S	G	S	G	T	D	F	T	L	7
			225			234			243			252			261			2
ATO	C AG	С.	AGT	CTG	CAA	CCT	GAA	GAT	TTT	GCA	ACT		TAC	TGT	CAA	CAG	AGT	T
		-																
I	S		S	L	Q	P	E	D	F	Α	T	<b>. Y</b>	Y	С	Q	Q	S	
												200			315			
			279			288	mm.c		297	ccc	NCC.	306		GAA		AAA	3 +	
AG	G GC	С	CCT	CAG	TGG	ACG	TTC		CAA		~						-	
R	 A	_	P	0	W	T	F	G	0	G	T	K	ν	E	I	K		
			CDR	~	•		-	-	_									

## Fig. 6a

#### LD2-1-VH sequence

		9			18			27			36						
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CT
Q	v	K	L	L	E	s	G	G	G	v	v	Q	P	G	G	s	L
AGA	CTC	63 TCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	стс	90 AGG	AGT	TAT		ATG		10 TG
 R		 s		v	 A	 s	<i>-</i> G	F		L	R	s	Y	G	M	н	W
												<del></del>		CDRI			<b>→</b>
ĠTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGC	135 CTG	GAG	TGG	GTG	GCT	TTT	ATA		TTT	
v	R	Q	Α	Б.	G	ĸ	<u>-</u>	L	E	W	v	A	F	I	W	F.	E
											100		<del></del>	207	CDR2		21
GGA	AGT	171 AAT	AAA	GGA	TAT	GTA	GAC	TCC	GTG	AAG	GGC	CGA	TTC		ATC	TCC	
G	 s	N									G	R	F	T	I	S	F
					- CDF	₹2 —					~ <del>~~</del>			261			2
GAC	AAT	225 TCC	AAG	AAC	234 ATG	GTC	TAT	CTG	CAA	ATG	AAC	AGC	CTG	AGA			_
D	N	s	к	N	М	v	Y	L	Q	М	N	s	L	R	Α	D	I
ACG	GCT	279 GTA	TAT	TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 CTT	CGG	GGA	315 ATC	AGC	AGA	32 TA
T	Α	v	Υ	Y	c	Α	R	E	ĸ	A		R					,
								<del></del>		200	260	- CD	R3	369			
AAC	TAT	333 TAC	CTG	GAC	342 GTC	TGG	GGC	351 AAG	GGG	ACC	ACG	GTC	ACC	GTC	TCC	TCA	3 '
N	_	Y CDR3			۸	W	G	K	G	T	T	V	T	٧	s	s	

## Fig. 6b

#### LD2-1-VL sequence

			•			18			27			36			45			54
	GTG		9			10	mc n	ccc	ጥርጥ	ccc	ACC	CCC	GGA	CAG	AGG	GTC	ACC	ATC
5'	GTG	GTG	ACT	CAG	CCA		ICA											
			т	Q	P	P	s	A	S	G	T	P	G	Q	R	v	T	1
									0.1			90			99			108
			63			12		* MC	O.T.	CCA	A CT	AAG	тат	GTA		TGG	TAC	CAG
	TCT	TGT	TCT	GGA	AGC	AAC	TCC	ATC										
	 s		s	G	3			I	L	G			Y	٧	Y	W	Y	Q
			<del></del>					c	DR1						<del></del>			162
			117			126			135			144		3 A III	153	CNC	ccc	
	AAA	CTC	CCA	GGA	ACG	GCC	CCC	AAA	CTC	CTC	ATC	TAT	AAG	AAT	GAI	CAG		
												Y						P
	K	L	₽	G.	T	Α	P	K	L	ы	1	1				CDR2		
												100	<del></del>		207	SDKZ		216
			171			180			189			198	ccc	ACC		GCC	TCC	
	TCA	GGG	GTC	TCT	GAC	CGA	TTC	TCT	GGC	TÇC	AAG	TCT						
									 G	s	K	s	G	Т	s	Α	S	L
	S	G	V	S	2	R	F	3	G	3		~						
	$\longrightarrow$					234			243			252			261			270
			225		CTC	234	TCC	GAG	GAT	GAG	GCT	GAC	TAT	TAC	TGT	GCA	CCA	TGG
	GCC	ATC	AGT															
	Α	I	s	G	ī	R	S	E	Đ	E	Α	D	Y	Y	С	Α	P	W
												206			315			324
			279			288			297	ccc	CCA	GGG	ACC	AAG	CTG		GTC	CTA
	GAT	GCC	AAC	CTG	GGT	GGC	CCG	GTG	TIC									
								v	F	G	G	G	T	K	L	T	٧	L
	D	Α	N	L						_								
			222		JK3 -				~									
	እ ርጥ	CAG	333															
				_														
	S	Q	P															

#### Fig. 7a

#### LD2-4-VH sequence

			9			18			27		cm.c	36	CNC	ccc	45	ccc	ጥርር	54 CTG
5'	CAG	GTG	AAA	CTG	CTC	GAG	TCG	GGG	GGA	GGC	GIG		CAG				TCC	
	Q	v	K	L	L	E	s	G	G	G	V	V	Q	P	G	G	S	L
	AGA	CTC	63 TCC	TGT	GÁA	72 GCG	TCT	GĞA	81 TTC	ACC	CTC		AGT		99 GGC		CAC	108 TGG
	 R		 s		 Е		 s	 G	F	T	L	R		s	_		н	W
			117			126	*		135			144						162
	GTC	CGC	CAG	GCT	CCT	GGC	AAG	GGG	CTG	GAG	TGG	GTG	GCA				TTT	
	. v	R	Q	Α	P	G	к	G	L	Е	W	v	A			CDD3	F	D
						100			100			198		<del></del>	207	CDRZ		216
	GGA	AGT	171 ATC	AGA	TCG	TAT	GCA	GAA	TCC	GTG	AAG	GGC	CGA	TTC		ATC	TCC	AGA
	 G	s	I	R								G	R	F	T	I	s	R
							12		242			252			261			270
	GAC	ACT	225 TCC	AAG	AAC	234 ACC	CTA	TAT	CTC	CAA	ATG	CGC	AGT	CTG		GCC	GAC	GAC
	D		 s	к	N	T	L	·Y	 L	Q	М	R	s	L	s	A	D	D
	ACG	GCT	279 <b>GT</b> G	TAT	TAC	288 TGT	GCG	AGA	297 GAC	AAG	GCG	306 GTT	CGG	GGA	315 ATT		AGG	324 TAC
	 T	 A	v	 Y	Y	c	Α	R			A	v		G			R	Y
									<del></del>			360	CD					
	AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351 AAA	.GGG	ACC	ACG				TCC	TCA	3'
	N	Υ	Y	M	D	v	W	G	к	G	Т	T	v	T	v	s	s	
			- CD	K3 —			<b>→</b>											

### Fig. 7b

#### LD2-4-VL sequence

		9			18			27			36			45			54
GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
~	 М		Q	s	P	s	s	L L	s	A	s	v	G	D	R	v	T
<b>ል</b> ሞር	АСТ	63 TGC	CGG	ACA	72 AGT	CAG	ACC	81 ATT	AGC	AGA	90 AAT	TTA	AAT	99 TGG		CAG	108 CAG
 I	 T	 C	 R							 R	 N	 L	N	 W	 Y	Q	Q
-	•	•	·`			_		_ cr	)R1 -				<del></del>	•			
AAA	CCA	117 GGG	AAA	GCC	126 CCT	AAG	стс	135 CTG	ATC	TAT	144 GCT	ACA	TCC	153 AGT	TTG	CAA	162 AGT
	 P											т	s	s	L	Q	s
														CDR2			<del></del> >
GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGA	тст	198 GGG		GAT	207 TTC	ACT	СТС	
 G	v	 P	 s	 R	 F	s	G	s	G	s	G	T	D	F	T	L	T
አጥሮ	דתת	225 AGT	СТА	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
		 S	 L	 Q	 P	 E	 D	 F			 Y			Q	 Q	 s	Υ
_	-	279		_	288			297			306			315			
ACT	ACC	CCT	TCG	TTC	GGC	CAA	GGG	ACC	AAG	GTG	GAA	ATC	AAA	3,			
T	т	P	s	F	G	Q	G	т	ĸ	v	E	I	К				
	- CD	R3 —	<del></del>														

### Fig. 8a

#### LD2-5-VH sequence

		9			18			27			36	~> ~		45	666	TCC.	
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	TTG	GTC	CAG					-
Q	v	ĸ	L	L	E	s	G	G	G	L	V	Q	P	G	G	S	
		63			72			81	•		90			99			1
AGA	CTC	TCC	TGT	GTA	GCG	TCT	.GGA	TTC	ACC	TTC	AGG	AGT	TAT	GGC	ATG	CAC	T
		 S			Δ				т	 F	R	 s	Y	G	М	н	
К	L	3	_				•					-		CDR1		<del></del>	
		117			126			135			144			153			
GTC	CGC	CAG	GCT	CCA	GGC	AAG	GGC	CTG	GAG	TGG	GTG	GCT	TIT	ATA	TGG	TTT	G -
													~		W		_
V	R	Q	Α	P							٧			1			_
		171			180			189			198			207			2
GGA	AGT	AAT	AAA	GGA	TAT	GTA	GAC	TCC	GTG	AAG	GGC	CGA	TTC	ACC	ATC	TCC	С
G	S	N									G	R	r	1	+	3	
								242			252	•		261			2
C T C	יחתת	225	א א כ	አክሮ	234	רייר	ጥልጥ	CTG	CAA	ATG	AAT	AGC	CTG		GCC	GAG	G
GAC	AAT			AAC												_	
D	N	s	ĸ	N	M	L	Y	L	Q	M	N	S	L	R	A	E	
		279			288			297			306			315			3
ACG	GCT	GTA	TAT	TAT	TGT	GCG	AGA	GAG	AAG	GCG	CTT	CGG	GGA	ATC	AGT	AGA	Т
T	Α	V	Y	Y	С	Α	R	E	K		L					ĸ	
								<del></del>			360	— СВ	кз —	369			
	m » ~	333	cmc	CAC	342 GTC	TGG	eec	351	GGG	GCC	ACG	GTC	ACC	GTC	TCC	TCA	
AAC	TAT	TAC	CTG	GAC	GIC	166											
N	Y	Y	L	D	V	W	G	ĸ	G	A	T	v	T	V	S	S	
		— CD	R3 -			<b>→</b>											

## Fig. 8b

#### LD2-5-VL sequence

		_			10			27			36			45			54
GTG	ATG	9 ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	ATA	GGC	GAC	AGA	GTC	ACC
 V	 М	 T	 Q	 s				L			s	1	G	D	R	V	T
»mc	እ <i>ር</i> ጥ	63 TGC	rcc	GCA	72 AGT	CAG	AGC	81 GTT	ACC	AGG	90 TCT	TTA	TAA	99 TGG	TAT	CAG	100 CA
T ATC			 R	 А	 s	Q	s	v	T	R	s	L				Q	_
AAA			AAA 	GCC	126 CCT	AGG	CTC	CDR 135 CTA  L	ATC		GCT	GCG			TTG  L	CAA	16 AG 
К	P		K					189 AGT			<del>←</del>	-		207			21
GGG  G	GTC  V	CCA  P	TCA  S	AGG  R				S						F	T	L	r
ATC	AGC	225 AGT	CTG	CAA	234 CCT	GAG	GAT	243 TTT	GGA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AAT	27 TA
 I	 s	 s	L	Q	P	E	D	F	G	T	Y	Y	С	Q	Q	N	'
AGG	ACC	279 CCT	CAG	TGG	288 ACG	TTC	GGC	297 CAA	GGG	ACC	306 AAG	GTA	GAĄ	315 ATC		. 3'	
R	т	Б	Q DR3	W	т	F	G	Q	G	Т	к	٧	E	. I	К		

### Fig. 9a

#### LD2-10-VH sequence

Y	N	Y	Y	L C	D DR3 -	٧	W	G	К	G 	T →	T	V	T	V	J	3
TAC	AAC	333 TAT	TAC	CTG		GTC		GGC	AAG	GGG	360 ACC	ACG	GTC	369 ACC			378 TCA
T	Α	v	Y	Y	Y	С	A	R					R - CDF	3 —			R
ACG	GCT	279 GTA	TAT	TAT	288 TAT	TGT	GCG	297 AGA	GAG	AAG	306 GCG	CTT	· CGG		ATC		
D	N	s	ĸ	И	М	v	Y	L	Q	М	N	s	L	R	A	D	D
GAC	AAT	225 TCC						243 CTG			252	AGC	CTG	261 AGA	GCC	GAT	270 GAC
G	s	N N	ĸ	G	Y	٧	D	S	v	. к	G	R	F	T	I	S	R
GGA	AGT	171 AAT	AAA	GGA	180 TAT	GTA	GAC	189 TCC	GTG	AAG	198 GGC			207			216
 V	 R	Q	Α	P	G	К	G	L	Ē	W	٧	A	F	I	W CDR2	F	D
GTC	CGC	117 CAG	GCT	CCA	126 GGC	aag	GGC	135 CTG	GAG	TGG	144 GTG			153			162 GAT
 R		s	c	v		s		F		·L		s ←	Y	G CDR1	М	H	W
AGA	CTC	63 TCC	TGT	GTA	GCG	TCT	GGA	81 TTC	ACC	CTC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	108 TGG
Q		к	L	L L	E	s	G	G	G	v	v	Q	P	G	G	s	L
CAG	GTG	9 AAA	CTG	CTC	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	54 CTG

## Fig. 9b

### LD2-10-VL sequence

		9			1 9			27			36			45			54
GTG	GTG	ACT	CAG	GAG	CCC	TCA	CTG	ACT	GTG	TCC	CCA	GGA	GGG	ACA	GTC	ACT	CTC
	 V		 Q	 E	P	 S	L		v		. P				v	T	L
ACC	TGT	63 GCT	TCC	AGC	72 ACT	GGG	GCA	81 GTC	ACC	AGG	90 GGT	TAC	TAT	99 CCA	AAC	TGG	108 TTC
т			s		T	 G	Α	v	T	R	G	Y		P	N	W	F
CAG	CAG	← 117 AAG	CCT	GGA	126			— CI 135 AGG			144		AGT		AAC		
 Q	 0	 К		 G				 R							N	K	K
_	TCC	171 TGG	ACC	CCT	180 GCC	CGG	TTC	189 TCA	GGC	TCC	198 CTC	CTT	GGG	207 GGC		GCT	216
	 S	 W	 T	 P	Α	R	F	s	G	S	L	L	G	G	К	A	A
CTG	<del>→</del> ACA	225 CTG	TCA	GGT	234 GTG	CAG	CCT	243 GAA	GAC	GAG	252 GCT	GAA	TAT	261 TAC	TGC	CTG	270 CTC
 L	<b>-</b> Т	 L			v			E									_
TAC	TAT	279 GGT	GGT	GCT	288 CAA	CTC	GTA	297 TTC	GGC	GGA	306 GGG	ACC	AAG	315 CTG		GTC	324 CTA
	Υ		 G	Α		 L	v	F									. <b>L</b>
CGT	CAG	333 CCC	1				,										
R	Q	P	-														

## Fig. 10a

#### LD2-11-VH sequence

		9			18			27			36			45			54
CAG	GTG	AAA	CTG	CTC	GAG	TCG	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTG
Q	v	ĸ	L	L	E	s	G	G	G	v	v	Q	P	G	G	s	L
AGA	CTC	63 TCC	TGT	GAA	72 GCG	тст	GGA	81 TTC	ACC	стс	90 AGA	AGT	TCT	99 GGC	ATG	CAC	108 TGG
 D		 S		 Е		 s	G	F	т	L	Ŕ	s	s	G	M	н	W
Γ.	ט		_	-	••	_	•	_				<del></del>		CDR1	. —	<del></del>	
GTC	CGC	117 CAG	GCT	сст	126 GGC	AAG	ĠGG	135 CTG	GAG	TGG	GTG	GCA			TGG		162 GAT
v	 R	Q	A	P	G	ĸ	G	L L	E	W	v			I	W W	F	D
					100			189			198		-	207	CDRZ		216
GGA	AGT	171 ATC	AGA	TCG	TAT	GCA	GAA	TCC	GTG	AAG	GGC		ŤTC		ATC	TCC	
<b>-</b>	 s		R							К	G	R	F	T	1	s	R
	·				- CDF	22				:	<del></del>			061			270
GAC	ACT	225 TCC	AAG	AAC	234 ACC	CTA	TAT	243 CTC	CAA	ATG	252 CGC	AGT	CTG	261 AGT	GCC	GAC	
D	 T	 s	К	Й 	т	L	Υ Υ	L	Q	М	R	s	L	S	A	D	D
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGA	297 GAC	AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGC	AGG	324 TAC
т	Α	v	Y	Y	c	A	R	D	К	A	v		G				Y
													R3 —				
AAC	TAT	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351 AAA	GGG	ACC	360 ACG		ACC	369 GTC	TCC	TCA	3'
N	Υ	Y	 М R3 —		V	W	G	к	G	T	T	V	T	V	\$	S	
		_ CD	KO —														

#### Fig. 10b

## LD2-11-VL sequence

•			_			1.0			27			36	B CT B		45			54
5 1	GTG	TTG	9 ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	ATA	CGA	GAC	AGA	GTC	ACC
J		 L		 Q	 s	 P	 s	 s	L	 S	Α	S	I	R	D	Ř	v	Ť
	ATC	ACT	63 TGC	CGG	GCA	AGT	CAG	AAC	81 ATT	GGC	AGT	90 TAT	TTA	AAT 	99 TGG 	TAT	CAG	108 CAC
	 I	 T		R	Α	s	0	N	I	G	S	Y	L	N	W	Y	Q	Н
	AAA	CCA	117 GGG					CTC	135 CTG		TAT	GCT	GTA	TCC	153 GCT		CAA	
		 P	 G	 T	A	p	ĸ					Α	v	S	A		-	S
									100			198	ACA		207			216
	 G												T				L	Т
	ATC	AGC	225 AGT	СТG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
													Y					Y
			070			288			297			306	CAG		315			
	 S	P		Y	T →	F							Q					

## Fig. 11a

#### LD2-14-VH sequence

		9			18			27						45			54
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTG
 Ω	v				 E			<b>-</b> G		v	v	Q	P	G	G	s	L
AGA	GTC	63 GCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TTC	90 AGG	AĄT	TTT	99 GGC	ATG	CAC	108 TG
 R		 A	c	v	Α	s	G	F	T	r	R			G CDR1	M	Н	W
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGG	135 CTG	GAG	TGG	144 GTG			153 ATT			162
v	<b>-</b>	Q	A	P	G	K				W	V	Α	F	Ξ	W CDD2		D
GCA	AGT	171 AAT	AAA.	GGA	180 TAT	GGA	GAC	189 TCC	GTT.	AAG	198 GGC			207 ACC			216
Α.	<b>S</b>	N	K	G	Y	G	D	S	v	K	G	R	F	T	٧	S	R
GAC	AAT	225 TCC	AAG		234			243 CTG			252 AAC	GGC	СТG	261 AGA	GCC	GAA	270 GAC
D	N	 S	 К	 N			Y		Q	M	N	G	L	R	A	E	D
ACG	GCT	279 GTA	TAT	TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGT	AGA	324 TAC
T	Α		Y	Y	С	A	R	E	К	A	V	R	G	I	S	R	Y
AAC	TAC	333 TAC	ATG	GAC	342 GTC	TGG	GGC	351 AAG	GGG	ACC	360 ACG	GTC	ACC	369 GTC	TCC	TCA	3,
N		Y CDR3				W	G	К	G	T	T	V	T	V	s	s	

## Fig. 11b

### LD2-14-VL sequence

			^			18			27			36			45			54	
ς,	GTG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTG	GGA	GAC	AGA	GTC	ACC	
-	 V		 T	 Q	 S	 P	 S	s	L	s	Α	S	V	G	D	R	V	T	
	ATC	ACT	63 TGC	CGG				AGC	ATT	ATC		AAT						108 CAG	
	I	T	С	R	Α	S		s				N	L	N	. W	Y	Q	Q	
	AAA	CCA	117 GGC	AAA	GCC	126 CCT		стс				144 GCT	GCA	TCC	153 AGT	TTG	CAA	162 AGT	
				 К	 A			L				Α		s	S	L	Q	S	
	·K	P	G	K	^							<del></del>				R2 -		<del></del>	
•	GGG	GTC	171 CCT	TCA	AGG	180 TTC	CGT	GGC	189 AGT	GGA	тст	GGG	AGA	GAT	TTC	ACT	CTC		
		 V					R	G	_	G			R	D	F	T	L	T	
	-			CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC	
	 V	т			Q	 P	E	D		Α		Y			Q	Q	S	Y	
	AGT	ACC	279 : CTG	TGG	, ACG	288 TTC	GGC	CAA	297 . GGG	ACC	: AAG	306 GTG	GAA	ATC	315 AAA				
	 S	т	L	 W	т	F		Q	G			v			К				
			- CD	R3 —		<del>-</del> →													

### Fig. 12a

## LD2-17-VH sequence

9 18 27  CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CT  Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  CDR1  117  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GG  V R Q A P G K G L E W V A F I W F I  GGA AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC GTG  G S N K G Y V D S V K G R F T I S L  GAC AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG  D N S K N T L Y L Q M K S L R A E  AAC GCT GTA TAT TAT TGT GCG AGA GAG GAG AAG GCG CTT CGG GGA ATC AGT AGA TG AAC ACG CTC TGG GAG GAG GAG AAG GCG CTT CGG GGA ATC AGT AGA ACC ATC TCC GTG AAG GCG CTT CGG GGA ATC AGT AGA ACC ATC TCC GTG AAG GCG CTT CAC ATC TCC GTG AAG AGC CTG GAG GAG GAG AAG GCG CTT TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC ACG GTC TCC TCA  AAC TAT TAC CTG GAC GTC TGG GGC AAG GGG ACC ACG GTC ACC GTC TCC TCA  AAC TAT TAC CTG GAC GTC TGG GGC AAG GGG ACC ACG GTC ACC GTC TCC TCA			_							_	T	ar.	• •	.1.	v	J		
9 18 27  CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CTC  Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAGA AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC CGC  G S N K G Y V D S V K G R F T I S L  GAC AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GCC  ACG GCT GTA TAT TAT TGT GCG AGA GAG GAG AAG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG TGC GTA TAT TAT TGT GCG AGA GAG GAG AAG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGT AGA TCC AGG AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGG AGG GCG CTT CGG GGA ATC AGT AGA TCC AGG AGA ATC AGT AGA TCC AGG AGG AGG GCC GTT CGG GGA ATC AGT AGA TCC AGG AGG AGG GCC CTT CGG GGA ATC AGT AGA TCC AGT AGT AGA TCC AGT	AAG	TA'	333 TAC	CTC	G GAC	342 GTC	TGG	. GGC	AAC	GGG	ACC	ACG	GTC	ACC	GTC	TCC		
9 18 27  CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CT  Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  TOTAL CORR CTG CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC CDR2  171  GGA AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC CGG AGT AAT CAC AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG TCC GAG GAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAC GCG GTG GTA TAT TAT TGT GCG AGA GAG AAG GCG CTT CGG GGA ATC AGT AGA TC AGT AGT AGA TC AGT AGA TC AGT AGT AGA TC AGT AGA TC AGT AGA TC AGT AGT AGA TC AGT AGT AGT AGA TC AGT						C	A	R	E	к	Δ	L	R	G	I	s	R	
27 CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CTG Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG R L S C V A S G F T F R S Y G M H W  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GTG GCT TTT ATA TGG TTT GAC TCC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAC TTC GAGA AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC GTG AAG GGC CGA TTC ACC ATC TCC GTG AAG GGC CGA TTC ACC ATC TCC GTG AAT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC GTG AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC GAG GAC AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC GAG GAG AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC GAG GAC AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG AAT TCC AAG AAC ACG CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG ACC ACT CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC AAT CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC AAT CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC AAT CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC AAT CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC AAT CTC TAT CTG CAA ATG AAG AGC CTG AGA GCC GAG GAG GCC AAT CTC TAT CTG CAA ATG AAG AGC CTG AGA ATC TCC AAC ATC TCC CTG CAA ATG AAG AGC CTG AGA ATC TCC AAC ATC TCC CTG CAA ATG AAG AGC CTG AGA GCC GAG GCC AAC TCC TAT CTG CAA ATG AAG AGC CTG AGA ATC TCC AAC ATC TCC CTG CAA ATC TCC CTG CAA ATC TCC AAC ATC TCC CTG CAA ATC TCC CTG CAA ATC TCC AAC ATC TCC CTG CAA ATC TCC CTG CTG CTC CTG CTG CTG CTG CTG C	ACG	GCT	279 r GT <i>F</i>	) A TAI	TAT	288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 CTT	CGG	GGA	315 ATC	AGT	AGA	3. T.
CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CTG  Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  CTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAC CTG GAG AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC CGC  GGA AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC CGC  G S N K G Y V D S V K G R F T I S L  CDR2  225  234  243  243  252  261  266  CGA GCC GAG GCC GCC																	E	
Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GA  V R Q A P G K G L E W V A F I W F I  GGA AGT AAT AAA GGA TAT GTA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC CG  G S N K G Y V D S V K G R F T I S I	GAC	TAA	225 TCC						243 CTG	CAA	ATG	252 AAG	AGC	CTG	261 AGA	GCC	GAG	
9 18  CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CT  Q V K L L E S G G G V V Q P G G S L  63 72 81 90 99 10  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  CDR1  117 126 135 144 153 16  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAC CTG GAG TGG GTG GTG GTG GTG GTG GTG GTG G					 G	Y	v	D	s	v								]
9 18  CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CT  Q V K L L E S G G G V V Q P G G S L  63 72 81 90 99 10  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  CDR1  117 126 135 144 153 16  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GCC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GCC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GCC AAG GGC CTG GAG TGG GTG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GCC AAG GCC CTG GAG TGG GTG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GCC AAG GCC CTG GAG TGG GTG GTG GTG GTG GTG GTG GTG G	-		_	ААА	GGA	180 TAT	GTA	GAC	189 TCC	GTG	AAG	198 GGC			207		TCC	2 : C
27 CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CT  Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W  117 126 135 144 153 166 177 GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  GTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  CTC CGC CAG GCT CCA GGC AAG GGC CTG GAG TGG GTG GCT TTT ATA TGG TTT GAG  117													Α	F	I	W	F	I
CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CT  Q V K L L E S G G G V V Q P G G S L  AGA CTC TCC TGT GTA GCG TCT GGA TTC ACC TTC AGG AGT TAT GGC ATG CAC TG  R L S C V A S G F T F R S Y G M H W	GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGC	CTG	GAG	TGG	144 GTG			153		TTT	16 GA
Q V K L L E S G G G V V Q P G G S L	<b></b>												s	Y	G	M		4
Q V K L L E S G G G V V Q P G G S L	AGA	CTC	63 TCC	TGT	GTA	72 GCG	TCT	GGA	81 TTC	ACC	TTC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	
9 18 27 CAG GTG AAA CTG CTC GAG TCT GGG GGA GGC GTG GTC CAG CCG GGG GGG TCC CT																		I
36 45 5	CAG	GTG	9 AAA	CTG	CTC	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	GGG	GGG	TCC	CT

## Fig. 12b

#### LD2-17-VL sequence

									27			36			45			54
د	CTC	እጥር	9 ACC	CAG	тст	CCA	TTC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
)				Q						 s			v	G	D	R	v	T
			•						81 ATT	AGG	AGT	90 TTT	TTA	AGT	99 TGG	TAT	CAG	108 CAG
		 T	c	R	Α	5	Q	N	I	R	S	F	L	S	W	Y	Q	Q
				<del></del>	GCC	126 CCT	AAG	CTC	- CI 135 CTG	ATC	TAT	144 GCT	GCA	TCC		TTG 		
	ĸ	P	- <b></b>	T	A	P	K	L	L	I	Y	A	A	s c	R DP2	L	Q	
	GGG	GTC	171 CCA	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GGG 		198	ACA	GAT	207 TTC	ACT	CTC	216
	 G	v	P	s	R	F	s	G	S	G	S	G	T	D	F	Т	L	1
	ATC	AGC	225 ACT	CTG	CAA	234 CCT	GAA	GAT	243 TTT	GCG	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	270 TAC
	 I	 S		 L	Q									С			.s	Y
,	AGT	GCC	279 : CCT	TGG	ACG	288 TTC	GGC	: CAA	297 . GGG	ACC	: AAG	306 CTG	GAA	ATC	315 AAA			-
	_	• • •	P CDR3		T	F	G	Q	G	T	к	L	E	I	К			

## Fig. 13a

## LD2-20-VH sequence

		^			18			27			36			45	•		54
CAG	GTG	AAA	CTG	CTC	GAG	TCT	GGG	GGA	GGC	GTG	GTC	CAG	CCG	GGG	GGG	TCC	CTC
 Q				 L	 E	 s	 G	G		v	v	Q	P		G	s	L
		63 TCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TCC	90 AGG	AGT	TAT	99 GGC	ATG	CAC	10: TG:
 R		 S		v	Α	s		F			R	S	Y		M	Н	
GTC	CGC	117 CAG	GCT	CCA	126 GGC	AAG	GGC	135 CTG	GAG	TGG	144 GTG			153			16 GA
· v	 R	Q	A	P				L	E				F		W CDR2	F	D 
GGA	AGT	171 AAT	AAA	GGA	180 TAT	GTA	GAC	TCC	GTG	AAG	198 GGC		TTC	207			21
<b>-</b> G	 S			 G	Y	v	, D	\$	٧	K		R			I	S	R
GAC	AAT	225 TCC	AAG	AAC			TAT	242			252 AAG	AGC	CTG	261 AGA	GCC	GAG	27 GA
 D	 N	 S	к	N		L		L			К				Α		ב
ACG	GCT	279 GTA	) A TAI	TAT	288 TGT	GCG	AGA	297 . ĢAG	AAG	GCG	306 CTT	CGG	GGA	315 ATC	AGT	AGA	32 T/
	 A				c			E	К	Α	L	R	G		S		· `
AAC	TAT	333 TAC	B C CTC	G GAC	342 GTC	: TGG	GGC	263			360			307		TCA	. :
	 Y	 Y										v		V			

## Fig. 13b

#### LD2-20-VL sequence

		9			18			27			36			45			54
STG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
v	 M	 T	Q	 S	P	 S	 s		s	A	s	v	G	D	R	v	T
		63	CGG	CCA	72	CAC	NGC	81	AGC	AGC	90 TAT	TTA	AAT	99 TGG		CAG	108 CAC
			 R				 S				Y		 И	 W	 Y		Q
Ι	T	С	· · ·											•			
AAA	CCA	117 GGG	AAA		120			125			144			153 AGT	TTG		16: AG
 К				 A			L L				A	Α	S	s	L	Q	S
								100						207			
GGG	GTC	171 CCA	TCA	AGG	TTC	AGT	GGC	AGT	GGA	TCT	GGG	ACA	GAT			CTC	AC
	v	р	S	R	F	s	G	S	G	s	G	T	D	F	T	L	T
ATC	AGC	225 AGT	СТG	CAA	234 CCT	GAA	GAT	243 TTT	GCA	ACT	252 TAC	TAC	TGT	261 CAA	CAG	AGT	27 TA
ī			L				D				Y				Q 	S	Y
AGT	ACC	279 CGA	TTC	ACT	288 TTC	GGC	CCT	297 GGG	ACC	AAA	306 GTG	GAT	ATC	315 AAA			
 S	T	R	F	T	F	G	P	G	T	К	v	D	I	K			

## Fig. 14a

#### LD1-6-17-VH sequence

CAG	GTG.	9 444	CTG	CTC	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCT	45 GGG	AGG	TCC	54 CTG
				L						v		Q	P	G	R	s	L
		<b>6</b> 2			72			81 TTT	ACC	TTC	90 AGT	AGC	TAT	99 GGC		CAC	108 TGG
				 A							s	s	Y	G	М	Н	W
GTC	CGC	117 CAG			GGC	AAG	GGG	CTG	GAG	TGG		GCA	GAT	ATA	TGG	TTT F	
٧	R	Q	Α	P	G	K	G	L	E	W	V	Α			W CDR1		_
GGA	GGT	171 AAT	AAA	CAT	180 TAT	GCA	GAC	189 TTC	GTG	AAG	198 GGC			207		TCC	216
	 G			н	Y	Α	D	F	v	• к	G	R	F	T	I	S	R
GAC	TAAT	225 TCC	AAG		224	CTG		243		ATG	252 AAC	AGC	CTG	261 AGA	GTC	GAG	270 GAC
D	~	 S		N	 T	v	Y	L	Q	М	N	S	L	R	V	E	D
ACG	GCT	279 GTG	TAT	TAC	288 TGT	GCG	AGG	297 GAT	TAC	TAT	306 AGC	GTT	ACT	315 AAG	AAA	CTC	324 AGA
 Т	Α	v	Υ	Y	С	A	R	D	Y	Y	S	٧	Т				R
CTC	CAC	333 TAC	TAC	TAC	342 TAC	ATG	GAC	351			360 AAA			369		ACC	378 GTC
L	н	Y		Y DR3		М	D	٧	₩ <b>→</b>	G	к	G	Т	T	V	T	V

TCC TCA 3'

\_\_\_\_

#### Fig. 14b

### LD1-6-17-VL sequence

		9			18			27			36			45		cmc	54
GTG .	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA		~
v							s					V			R		
ATC	ACT	63 TGC	CGG	GCA	AGT	CAG	GGC	81 ATT	AGA	AAT	90 GAT	TTA	ACC	99 TGG	TAT	CAG	108 CA/
 I	 Т		 R	A	 s	0	G	I	R	N	D	L	T		Y	Q	Q
AAA	CCA	117 GGG	<b>←</b>	GCC	126 CCT	AAG	СТС	CDR	ATC	TAT	144 GCT	GCA	TCC	153 AAT			
ĸ	P	G	к	A	P	K	Ļ	.L	I	Y	Α	Α	S	ODR2	L —		
			TCA	AGG	180 TTC	AGC	GGC	189 AGT	GGA	TCT	198 GGC	ACA	GAT	207 TTC	ACT	CTC	AC
 G	v	P	S	R	F	s	G	S	G	S	G	T	D	F	T	Ŀ	Т
ATC	AGC	225 AGC	CTG	CAG	234 CCT	GAA	GAT	TTT	GCA	ACT	252 TAT	IAC	101	CTA	CAA	GAT	27 AA
	 S						D							L	Q	D	N
AAT	TTC				000		CAG	207	i		306			315	1		
-		P	Y		F	G	Q	G	т	к	L	E	I	K			

### Fig. 15a

## LD1/2-6-3-VH sequence

5'	CAG	GTG	9 AAA	CTG	CTC	18 GAG	TCT	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	54 CTG
	0		 к.			 E	 S	 G			v	v	Q	P	G	G	S	L
	_	GTC	63 . GCC	TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TTC	90 AGG	AAT	TTT	99 GGC	ATG	CAC	108 TGG
	 R	 v	 A				 \$		F			R	N	F	G	M	Н	W
		CGC	117 CAG	GCT	CCA	126 GGC	ĄĄG	GGG	135 CTG	GAG	TGG	144 GTG	GCT		CDR1 153 ATT			162 GAT
	 V	 R	 Q	 A	 P	 G			L		W		Α	F	I	W	F	D
		-	-	AAA	GGA	180 TAT	GGA	GAC	189 TCC	GTT	AAG	198 GGC			207		TCC	216
			 N		 G	Y	<b>-</b>	D	S	ν	К			F		V	S	R
							R2 CTC					252 AAC	GGC	CTG	261 AGA	GCC	GAA	270 GAC
			 S			T	L	Υ	L	Q		N		L	R	A		D
	_					288 TGT	GCG	AGA	297 GAG	AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGT	AGA	324 TAC
						c	Α	R	E	К	Α		R	G	I	S	R	Y
	AAC	: TAC	.333 TAC	S E ATO	GA(	342 GTC	TGG	GGC	351 3AA	GG		360	1		369	TCC	TCA	3'
	N	Y	Y CDR	М	Đ		 W	G		G	Т		٧			S	s	

### Fig. 15b

## LD1/2-6-3-VL sequence

									27			36			45			54
			9		тст	18	mac	mcc	CTC	ጥርጥ	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC
5 '	GTG	ATG	ACC	CAG	TCT	CCA	TCC	100										
									L	S	Α	s	v	G	D	R	V	T
	V	M	T	_	S													
						7.0			R 1			90			99			108
			63		GCA.	12	CNC	NGC	ስጥጥ	ATC	AGA	TAT	TTA	AAT	TGG	TAT	CAG	CAC
	ATC	ACT	TGC	CGG	GCA.	AGT	CAG	700										
					Α		0	S	Т	I	R	Y	L	N	W	Y	Q	Н
	I	т	C	ĸ	А	3	×	~	C[	191 –								
				<del></del>					3 3 F			1 4 4			153			162
			117		GCC	120	N A C	ርጥሮ	CTG	ATC	CAT	ACT	GCA	TCC	AGT	TTG	CAA	AGT
	AAA	CCA	GGG	AAA														
		<del>-</del>				<b>D</b>	ĸ	7.	L	I	Н	T	Α	ج.	S	L	Q	S
	К	P	-	K								4			<del>-</del> ເນ	R2 -		<del></del>
						100			189			108			207			216
			171		AGG	180	እ ርጥ	cec	AGT	GTA	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC
	GGG	GTC	CCG	TCA														
							9	G	S	V	S	G	T	D	F	T,	L	T
	G	V	P	S														
			205	•		234			243			252	መእር		261			270
			225	CTC	. CAA	ርርጥ ርርጥ	GAA	GAT	TTT	GCA	ACT	TAC	TAC	TGT	CAA	CAG	AGT	TAC
	ATC	AGC	AGI															 Y
			s	L	0	Þ	E	D	F	Α	T	Y	Y	С	Q	Q	S	1
	I	S			_										<del></del>			
			279	1		288			297	,		306			315			
	N C M		613	, - ጥልር	ACT	ጥጥፕ	GGC	CAG	GGG	; ACC	: AAG	CTG	CAG	ATC	: AAA	, 3.		
	ACT	ACC														•		
		T	P	Y	т	F	G	Q	G	T	K	L	Q	·I	K			
	-	-	CDR3	_														
			CDK2		,													

### Fig. 16a

## LD1/2-6-33-VH sequence

CAG (	cmc	9	CTG	ርፐር	18 GAG	тст	GGG	27 GGA	GGC	GTG	36 GTC	CAG	CCG	45 GGG	GGG	TCC	CTG
	 V			 L	 E	 S		 G		v	v	Q	P	G	G	s	L
AGA			TGT	GTA	72 GCG	тст	GGA	81 TTC	ACC	TTC	90 AGG	AAT	TTT 	99 GGC 	ATG		108 TGG
						 S		F			R	N	F	G CDR1	M	H	W
						AAG	GGG	135 CTG	GAG	TGG	144 GTG	GCT	TTT 	153 ATT	TGG	TTT	162 GAT
	 R	Q	Α	P	G	K	G	L	E	W	V		F	I 	W CDR2	F	
GCA	AGT	171 AAT	<b>AAA</b>	GGA	180 TAT	GGA	GAC	189 TCC	GTT	AAG	198 GGC			207			216 AGA
	 S	 N	 K								G					S	R
			AAG		- CDI	R2					<del>}</del>			261	GCC	GAA	270 GAC
			 K	 N	т	 L	<b>Y</b>		Q		N		L	R	Α	E	D
_			TAT	TAT	288 TGT	GCG	AGA	297 GAG	, AAG	GCG	306 GTT	CGG	GGA	315 ATT	AGT	AGA	324 TAC
 T				Y				E	к	Α	٧	. <b>R</b>	G		S	R	Y
AAC	TAC	333 TAC	B C ATC	G GAC	342 GTC	TGG	GGG				260	1	ACC	369 GTC	TCC	TCA	3'
N	_		м 3 —		∨ 	W	G	К	G	T	T	V	T	٧	S	S	

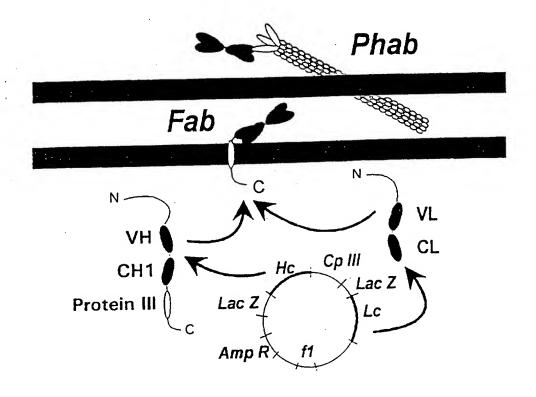
#### Fig. 16b

### LD1/2-6-33-VL sequence

			9	CAG	m.c.m	18	שרר.	ጥጥር	27 CTG	тст	GCA	36 TCT	GTA	GGA	45 GAC	AGA	GTC	ACC
5'	GTG	ATG	ACC	CAG	TCI													
	v	 М	 T	Q	S	P	S	F	L	S	Α	S	V	G	D	R	V	T
		3 CM	63	CGG	CCA	72 AGT	CAG	AGC	81 ATT	ATC	AGA	90 TAT	TTA	AAT	99 TGG	TAT	CAG	108 CAC
	ATC		 C	 R				5	T	I	R	Y	L	N	W	Y	Q	н
	.1	•													153			162
	200	CCA	117 GGG	AAA	GCC	126 CCT	AAG	CTC	135 CTG	ATC	CAT	144 GCT	GCA	TCC	AGT	TTG	CAA	
		 P	 G		 A	 P					Н	Α	Α	S	S	L	Q	S
	••	-																216
	GGG	GTC	171 CCG	TCA	AGG	180 TTC	AGT	GGC	189 AGT	GTA	TCT	GGG	ACA	GAT	TTC	ACT	CTC	ACC
	 G			 S	R	F	S	G	s	V	, s	G	Т	D	F	T	L	Т
												252			261 CAA	CAG	AGT	270 TAC
				 L	<b></b>					Δ	т	Y	Y	С	Q	Q	S	Y
	1	S																
	N C T	י ארו	279	) G TAC	: ACT	288 TTT	GGC	CAC	297 G GGG	ACC	AAG	306 CTC	CAG	ATC	315 AA/		,	
		T											Q			-		
			-	· —														•

Fig. 17

# The pComb3 Expression System



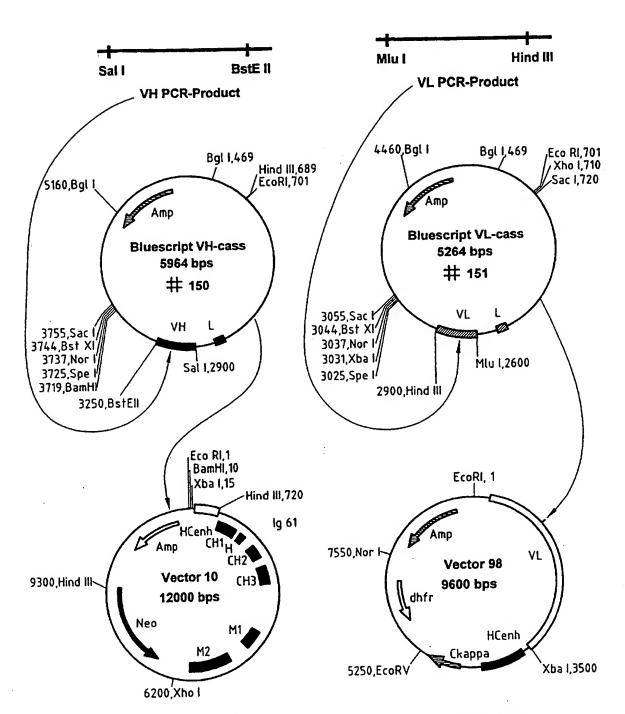


FIG. 18

FIG. 19

#### INTERNATIONAL SEARCH REPORT

tn. atlanal Application No PCT/EP 97/03253

			<del>_</del>	
A. CLASSI IPC 6	FICATION OF SUBJECT MATTER C12N15/13 C12N15/62	C07K16/34	A61K39/395	G01N33/80
According to	o International Patent Classification (IPC) or to both nati	onel classification en	d IPC	
B. FIELDS	SEARCHED			
Minimum do IPC 6	cournentation searched (classification system followed C12N C07K A61K G01N	by classification sym	iols)	R-
Documental	tion searched other than minimum documentation to the	extent that such doc	uments are included in the	fields searched
Electronia d	ata base consulted during the international search (nar	ne of data base and,	where przotical, search ter	rms used)
C. DOCUME	ENTB CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where approprie	ite, of the relevant pa	asages	Relevant to claim No.
X	SIEGEL D. L. & SILBERSTEIN "Expression and character recombinant anti-Rh(D) and filamentous phage: a model isolating human red blood by repertoire cloning" BLOOD, vol. 83, no. 8, 15 April 1 pages 2334-2344, XP0006090 cited in the application see the whole document	ization of tibodies on lsystem for cell antib		11-13.
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"A" docume conside "E" earlier de filing de "L" documes which is citation "O" documes later the Date of the a	nt which may throw doubts on priority claim(s) or is clad to establish the publication date of snother is or other special reason (as specified) intrefering to an oral disclosure, use, exhibition or neans in published prior to the international filing date but an the priority date claimed sound completion of the international search	Or of or	r priority data and not in on ted to understand the prinoi vention ourset of particular relevan unnot be considered novel or volve an inventive stap who oursent of particular relevan unnot be considered to invo- oursent to combined with o sents, such combination bei the art. oursent member of the earn to of mailing of the internati	or cannot be considered to in the document is taken alone toe; the claimed invention the an inventive step when the the or more other such doou- ing obvious to a person skilled to patent family
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NEUM SING TO	nating address of the ISA  European Patent Office, P.B. 5818 Patentiaen 2  NL - 2280 HV Rijewijk  Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  Fax: (+31-70) 340-3016	Au	Müller, F	

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